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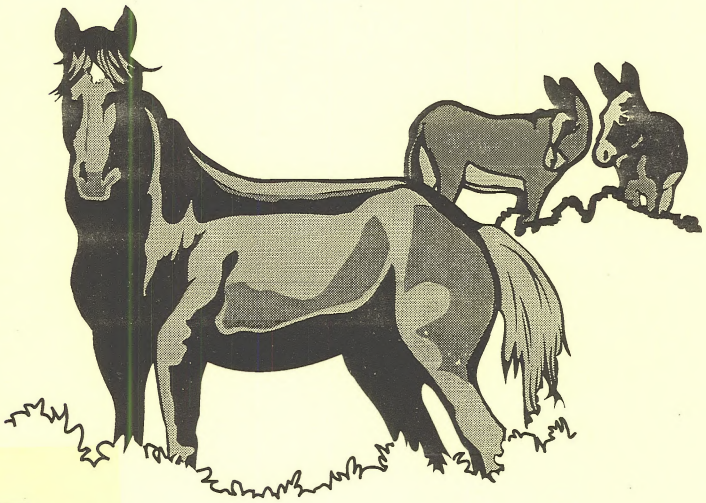
WILD HORSE & BURRO FERTILITY MANAGEMENT POLICY AND PROCEDURES TASK GROUP

FINAL REPORT



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BUREAU OF LAND MANAGEMENT
JUNE 1992

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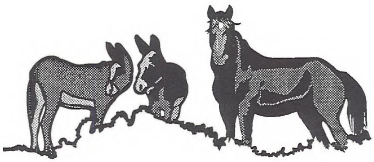
WILD HORSE and BURRO FERTILITY MANAGEMENT

POLICY and PROCEDURES TASK GROUP, FINAL REPORT

April 21, 1992

Summary

Fertility control is a potentially powerful tool for managing wild horses and burros. This approach to management is identified in the Strategic Plan for Management of Wild Horses and Burros On Public Lands, and is authorized through the Wild Free-Roaming Horse and Burro Act of 1971, as amended, and the Department of the Interior and Related Agencies Appropriations Act of 1992. A number of new contraceptive technologies can now be applied to wild horse populations including selective removals, sterilization, steroid hormones, and immunocontraception. Based on analysis of current technology, selective removals based on age or sex parameters, and immunocontraception appear to provide potential fertility control which is effective, safe, reversible, and cost effective. These two techniques are recommended for implementation in a pilot study proposed for the Antelope/ Antelope Valley herd management areas. Implementation of a pilot project will include methods for monitoring, tracking, and evaluation. Specific monitoring methods were reviewed to ensure animal safety and health throughout the study, while providing information concerning the effectiveness of the treatments. Although selective removals and immunocontraception are recommended techniques for incorporation in a pilot project, none of the techniques are rejected from further consideration. The rapidly advancing nature of anti-fertility research necessitates that yearly reviews be conducted to evaluate available technologies and their potential for implementing the Strategic Plan for Management of Wild Horses and Burros on Public Lands.



WILD HORSE and BURRO FERTILITY MANAGEMENT
POLICY and PROCEDURES TASK GROUP, FINAL REPORT

INTRODUCTION:

The use of fertility control has been identified in the Strategic Plan for Management of Wild Horses and Burros on Public Lands as a management alternative for reducing the rate of wild horse population growth. Managing wild horses and burros using fertility control measures is authorized by the following legislation:

* Wild Free-Roaming Horse and Burro Act of 1971 (Public Law 92-195), as amended by The Federal Land Policy and Management Act of 1976 (Public Law 94-579) and The Public Rangelands Improvement Act of 1978 (Public Law 95-514) Section 3 (a) "The Secretary shall manage wild free-roaming horses and burros in a manner that is designed to achieve and maintain a thriving natural ecological balance on the public lands. He shall consider the recommendations of qualified scientists in the field of biology and ecology..."

  Section 3 (b)(1) "...determine whether appropriate management levels should be achieved by the removal or destruction of excess animals, or other options (such as sterilization, or natural controls on population levels). In making such determinations the Secretary shall consult with...such other individuals whom he determines have scientific expertise and special knowledge of wild horse and burro protection, wildlife management and animal husbandry as related to rangeland management."

* Department of the Interior and Related Agencies Appropriations Act of 1992 (Public Law 102-154) 43 USC 1474a, Conference Report 102-256, Making Appropriations for the Department of the Interior and Related Agencies for the Fiscal Year Ending September 30, 1992, and for Other Purposes. Monies specifically directed for research in the wild horse and burro program are: Amendment No. 1: "...$100,000 for a population model, $200,000 for immuno-contraception research, $100,000 for vegetation monitoring, $250,000 for mapping and census data, and $200,000 for fertility control, all in Nevada and all in the wild horse and burro program."

Finding socially, biologically, and economically acceptable solutions for the disposition of excess animals has been difficult. Fertility control offers the management alternative of reducing the rate of population growth, and in doing so reducing the number of excess animals to be removed.

The Pilot Fertility Project Team was established with the intent of addressing how fertility control management could be initiated for wild horses. The group consists of representatives from a variety of disciplines and interested parties. Group members met to discuss general concerns. Two sub-committees were developed with the purpose of evaluating: 1. preliminary methods of application and/or treatment, 2. procedures for monitoring, tracking and evaluation. The final Wild Horse Fertility Control Study Recommendations is a compilation of the two sub-committee reports. The recommendations are based on the current technology.

I. GROUP ONE SUB-TASK DESCRIPTION: PRELIMINARY METHODS OF APPLICATION/TREATMENT

Evaluate available fertility control methods and develop guidelines for their application to reduce the rate of increase in wild horse populations.

II. DISCUSSION

The use of fertility control has been identified through the Strategic Plan for Management of Wild Horses and Burros on Public Lands as a management alternative for reducing the rate of increase of wild horse populations. Although fertility control has not been a widely prescribed management alternative for

populations. Although fertility control has not been a widely prescribed management alternative for inhibiting wild horse and burro population growth, sterilization and selective removals have been used to eliminate physical abnormalities and improve the adoptability of excess animals. The potential for expanding management into controlling the growth rate of wild horse populations has resulted from recent advances in contraceptive research. Several methods now exist that are safe and humane, reversible, and effective in slowing wild horse reproductive rates. The task group identified that the fertility control methods which are currently available for field application should be evaluated for their adaptability to the management of all Nevada's herd management areas (HMAs), as well as, to the pilot fertility control project. The criteria used to select a fertility control procedure is to be based on several premises: 1. the method must be effective in reducing reproduction in wild horse populations, with effectiveness of two or more seasons constituting the ideal, 2. the procedures must be safe and humane for the animals receiving it, including pregnant animals, and should minimize disruption to herd dynamics, 3. the effects of the treatment should be reversible, 4. the fertility control measure should be cost effective, balancing the measure of control, duration of effectiveness, with cost per animal treated.

Applying fertility control to the management of wild horses has raised concerns regarding numerous facets of the procedures. The Task Group identified the following concerns:

A. Consider the two HMAs (Antelope and Antelope Valley) as one unit due to the interaction between HMAs or consider other areas.

B. What amount of participation will be provided by the University of Nevada, Reno (UNR)?

C. Conduct a literature search considering all forms of fertility control (unpublished research).

D. Identify new constraints and requirements as imposed by the fiscal year 1993 WH&B requirements gather contract.

E. Develop alternatives for fertility control or population control.

F. Consider field treatment vs Palomino Valley Center (PVC) treatment, i.e., shipping long distance portable vet lab, holding facility, etc.

G. Identify selective removal target group (age, sex, % treated, etc.).

H. Provide qualified personnel to implement projects (implants/shots) eg., veterinarians (vets) vs. WH&B specialist.

I. Provide a sample of treated animals held at PVC for control on effects and available for UNR studies.

J. Has age in HMA already been manipulated? Consider in pilot project, should we suspend policy to remove "selectivity"...gate cuts?

K. Consider intensive study vs. field study. A literature search will be one of the easiest/quickest parts of the study.

L. Will the method chosen transmit the drug to the foal?

M. How will mares be selected: what is the target group?

N. Will permanent fertility control be used more than physical abnormalities?

O. Will vet technicians or vets do field procedures?

P. Surgical implants are a common procedure.

Q. Balistavet™ is a new inoculation method which may be available in time release within one application.

R. Equipment used should only be for use in the "field" to avoid transfer of disease (don't expose to "carrier" horse).

S. For age selective removals, target mares, not stallions: look at studies/facts about ages mares reproduce, most fertile, mortality at different ages, will age affect stress/abandonment of foals? Even age population is ideal.

T. Portable vet labs easily available, upgrade portable chutes. (Oklahoma sanctuary has a good example.)

U. Timing of injection, alteration of foaling season, minimize animals handling.

V. Selective removals should target sex, quality (deformations, etc.), and age.

W. What are the variations in treatments between stallions and mares: surgery, no surgery, death loss, rehabilitation time from surgery, qualified personnel, testing % of animals (blood, etc.), ?

X. Develop guidelines for each alternative method of control for field implementation.

Y. Develop guidelines for selecting animals to be treated.

Z. What methods are available and what factors are to be considered in selecting a method(s)?

Several additional concerns have arisen since the January 16, 1992, Wild Horse Fertility Project Team Meeting.

AA. Should the treated animals should be quarantined before release back into the herd?

BB. How should the holding facility be designed and should it be located within close proximity to the capture site?

CC. Should efforts be made to preserve band integrity upon animal release back into the HMA?

DD. What factors will be considered in locating a release point back into the HMA (i.e., water, forage, concentration of animals)?

The concerns focus on the criteria for selection of a fertility control method (concerns E, L, N, V, X, Z), selection of targeted animals (concerns G, J, M, S, U, Y), how and who would be administering the chosen method (concerns H, O, P, Q, W), and the handling of animals during and after treatment (concerns F, I, K, R, T, AA, BB, CC, DD). Concerns A, B, C, and D do not represent procedural concerns to be governed by policy or manual guidance. As such, they will not be addressed in this analysis.

III. OPTIONS/ANALYSIS

The selection of a fertility control method is limited by the existing technology. There are currently three principal types of fertility control which are applicable to wild horse management. The first is selective removal to alter normal herd structures and disrupt breeding patterns, second is permanent sterilization, and third is temporary inhibitors utilizing either surgical implants or intramuscular injection of control agents to block pregnancy or prevent fertilization. The latter group is represented by two methods which have been shown to be effective on wild horse populations: steroid implants, and immunoantifertility agents.

A. ACTIONS COMMON TO ALL ALTERNATIVES

Evaluating available fertility control methods and developing procedures for implementing those methods involves certain actions which are common to all available methods.

1. Capture animals following current Nevada capture policies and procedures.

2 Gathers and treatments will not occur during the peak foaling season time periods of March 1 through June 30.

3. Sort animals by sex and age.

4. Remove excess animals by identified target group (age, sex). Only animals aged 1-3 (target group ages may be modified depending on population modeling) will be removed from the herd management areas should an excess be identified. Limiting removal of excess animals to younger age groups will increase their adoptability.

5. Animals to be released back into the herd will be held until termination of the operation to minimize the added stress of recapture.

6. Move animals to be returned to the range to holding facilities away from capture/shipping facilities. Holding facilities may or may not be required depending on the number of trap sites to be used, the number of animals to be removed, and the distribution of animals throughout the gather area.

7. Animals to be returned to the range, which are in holding facilities servicing more than one trap site, should be marked in correlation to the trap site where they were captured and if possible by band to maintain band integrity. These animals, as marked, should then be released in the general area where they were captured, and if possible, released by band. Mares with foals should be separated from other animals and released last to ensure they remain paired. Releasing animals should take into consideration fences, geologic barriers, etc.

8. Excess animals should be shipped as soon as possible to avoid contracting diseases associated with confinement.

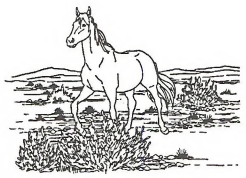
9. Release animals as each trap site operation is completed or upon completion of all gather operations. Effort should be made to minimize holding time to reduce disease exposure to communicable diseases.

10. All methods will require additional monitoring to determine effects on animal health, herd structure, population dynamics, and the effectiveness of the treatment. The method chosen will determine the additional monitoring requirements.

B. SELECTIVE REMOVAL

Selective removal is used to adjust the existing population dynamics of a herd in order to disrupt normal breeding behavior and limit productivity. Selective removals may be used to adjust either the age structure or the sex ratio within the herd.

Altering the age structure of the herd is aimed at reducing the number of animals in the primary breeding age groups. The age structure of wild horse populations is weighted heavily toward younger animals. Data collected during removal operations indicate that approximately 45 percent of the total population is represented within age groups 1 through 3 with 90 percent in groups 1 through 9. Wild horse mares are able to first conceive at age 2 and continue producing into their late teen years. Peak fertility is realized during ages 3 through 9. After that period, mare cycling becomes less regular and more dependent on fluctuating environmental and physiological factors which serve



to restrict reproduction. Males are able to first breed at age 1 and will continue to do so until death or preclusion through social interactions within the herd. Peak reproductive years for males are between ages 4 through 9. After that time, physical decline and loss of social dominance reduce participation in reproduction. Targeting of young age wild horses would reduce the number of animals of prime breeding age while allowing for the continued, although slowed, growth of the herd.

A proposed age specific selective removal program would target removing approximately 90 percent of the 1 to 3 year old animals with repeated removals every 3 years. Population modeling indicates that repeated treatments may be able to slow foal recruitment from 18 percent of the total population down to 10 percent. Normal age distribution would be achieved after approximately 12 years, following initial treatment. Altering the age structure of the population could impact herd behavior. As the population ages and fewer animals are available to fill dominant roles in the social structure, older animals would continue to dominate and reduced competition for dominance could result in bands containing larger numbers of animals.

Selective removal of wild horses to modify sex ratios would provide similar results to those described in selectively removing age groups. Capture data shows wild horse populations are approximately 45 percent male and 55 percent female. The skewed proportions are attributable to more males in the older age groups. As with age specific selective removal, the premise of altering the sex ratio is to reduce the number of breeding animals. By increasing the proportion of males to females, fewer animals are available to reproduce. Population modeling has shown that a ratio of 70 males to 30 females may be able to slow foal recruitment from 18 percent of the total population down to 12 percent.

A proposed selective removal project to alter sex ratios would target mares aged 1 through 6. Initial population modelling indicates that a large proportion of the mares would require removal to successfully limit reproduction through sex ratio manipulation. Ages 1 through 6 represents approximately 80 percent of the female population. Male populations would remain intact.

Each of the selective removal procedures would be reversible over time. Discontinuing removals would allow the population to achieve a normal balance. The time required to achieve a normal age structure or sex ratio would be dependent on the size of the population and number of treatments administered prior to termination.

1. Procedures

Sort animals by sex and age. Remove excess animals by identified target group (age, sex). Only animals aged 1-3 (target group ages may be modified depending on population modeling) will be moved from the herd management areas should anexcess be identified. Limiting removal of excess animals to younger age groups will increase their adoptability.

2. Analysis

The following analyzes the positive and negative aspects of selective removals:

a. Positive

(1) No additional cost. There would be no additional costs associated with selective removal gathers above those experienced during 1991-1992.

(2) No additional animal handling. The capture process already requires the aging and sexing of captured animals at the trap site.

(3) No additional animal stress. The current capture procedures would be followed.

(4) Reversible and self-correcting. The treatments are easily reversible through natural reproduction and discontinuation of the treatments. The animals would not need to be captured or handled to reverse the process.

(5) No increased time requirement. The animals would not have to be held for treatment or observation following treatment. The animals would only be held

until completion of the gather. This would reduce treatment cost and animal stress.

(6) No medical waste. There would be no accumulation of waste resulting from medical procedures.

(7) No introduction of drugs into the environment. Since there are no implants or injections involved in the procedure, no chemicals would be introduced into the food chain.

(8) There would be no need to mark animals for tracking.

b. Negative

(1) Short term. For the treatment to be successful, it must be repeated every three years for approximately three cycles. The term between cycles may be lengthened only by removing animals from more age groups (i.e., removal of one to four year old animals would create a four year cycle of removals).

(2) Disruptive to the social structure. The treatments will disrupt the social structure of the herd. Either age or sex specific removals will skew the natural balances. Current information regarding "normal" herd behavior is limited, thus limiting the ability to predict the effects of altering the natural balance. Band integrity could also be affected resulting in potential impacts to reproduction.

C. STERILIZATION

Sterilization is used to achieve as many years of reproduction inhibition as possible through a single treatment. Either stallions or mares may be targeted for sterilization to control reproductive rates.

Studies by the University of Minnesota have produced mixed results when targeting stallions. Field trials vasectomizing dominant stallions show that the fertility control is only effective over a short period of time. Stallion dominance is transitionary, changing between years. The prospect of sterilization as a one time population control measure is substantially diminished unless subordinate males are also treated.

For vasectomy to be effective several conditions must be met:

- The dominant stallion must do all, or the majority of, the breeding in the band. The role of subordinate males in siring foals is unknown, especially when the dominant stallion is vasectomized and sterile.

- Bands must be relatively stable with minimal exchange of animals between bands. Significant exchange of intact stallions would render the technique ineffective.

- Vasectomized stallions must retain their dominance for several years even when not intact. If dominance is transitionary, then one-time herd sterilizations will only provide short-term effectiveness.

Although the cost of individual vasectomy is not prohibitive, the number of animals which would require sterilization could become costly.

Many of the concerns limiting the effectiveness of vasectomizing stallions would not be applicable with mares. Population modeling can predict the number of animals which would require treatment to suppress reproductive rates within a population. The exchange of mares between bands would not affect the inhibition of reproduction. Movement of animals from one herd management area to the next would complicate the modeling process. The process would have to be repeated after approximately three years to treat recruited animals.

Selection of stallions or mares for treatment would result in varying degrees of effectiveness. Of concern in either case is the non-reversible nature of the treatment. The genetic pool in each population exists in a dynamic state with each reproductively active animal influencing the diversity of the pool. Limiting the reproduction and transferring of genetic variety to a select group of animals within each population would impact the natural selection process.

1. Procedures

Sterilization of mares could be completed using one of several methods to surgically alter the female reproductive organs.

a. Stallion Sterilization

(1) Vasectomy - Stallions would be anesthetized and restrained in dorsal recumbency. The scrotal area would be surgically prepared and an incision made at the head of each testicle. The vas deferens is located and a segment of ap proximately one inch is removed. It is necessary to ligate both cut ends of the vas deferens. Incisions are sutured appropriately, preferably with absorbable material. Tetanus antitoxin and a long acting antibiotic are administered.

(2) Epididymectomy - This surgical procedure has not been reported in horses, however, it is used successfully to sterilize bulls and rams. Anesthetic, restraint and surgical prep are the same as vasectomy. A small incision is made through the skin into the tail of the epididymis. A majority of the tail of the epididymis is removed. The incisions are not sutured to allow for proper drainage. Tetanus antitoxin and a long acting antibiotic are administered.

(3) Castration - Anesthetic, restraint and surgical prep is the same as for the vasectomy. The testicles are removed through two skin incisions. An emasculator is used to sever the spermatic cords and in older horses or at the discretion of the surgeon, the cord would be ligated. Incisions are not sutured to allow for proper drainage. Tetanus antitoxin and a long acting antibiotic are administered.

NOTE: The three surgical procedures to sterilize stallions would require the same materials: anesthetic, soft cotton rope, soap, skin antiseptic, surgery pack, sutures, tetanus antitoxin, antibiotics and various needles and syringes. The length of these operations would vary according to the skill and experience of the surgeon. The anesthetic recovery time will vary with the type and amount of anesthetic used. Healing time for all these operations would be approximately two (2) weeks. These are considered as minor surgical procedures and would not require any special handling post surgery. All of these operations could be performed in the field.

b. Mare Sterilization

(1) Ovariectomy (Spay) - This surgical procedure to remove the ovaries can be accomplished via two different approaches (vaginally or through the side of the abdomen). With both of these approaches it is simpler to perform if the mare remains standing. Therefore, these operations could be done in a squeeze chute with belly bands using a tranquilizer and local anesthetic. After the proper surgical preparation, incisions are made on the side of the abdomen or through the vaginal wall. The ovaries are located and removed with an ecrauser. Incisions are sutured appropriately, preferably with absorbable suture materials. Tetanus anti toxin and a long acting antibiotic are administered.

(2) Salpingectomy (Tubal ligation/separation) - This operation involves removal of a portion of the oviduct and could be accomplished through an abdominal incision or with endoscopy. Again the preferred method is to have the mare standing, so restraint and anesthetic would be the same as for ovariectomy. After entering the abdominal cavity, the oviduct is located and a segment of approximately one inch is removed. Both cut ends should be ligated. Invasions are sutured

appropriately, preferably with absorbable suture material. Tetanus antitoxin and a long acting antibiotic are administered.

NOTE: These surgical procedures in the mare are more invasive than any of the stallion surgeries, therefore, mares would have to be held for at least two (2) weeks after surgery for observation. If no complications develop, they could be released back to the range after this observation period. The materials needed for the surgical procedures in the mare include: squeeze chute with belly bands, anesthetic, tranquilizer, hair clippers, soap, skin antiseptic, surgery pack, ecrauser (for spay only), sutures, tetanus antitoxin, antibiotics and various syringes and needles. The length of these operations will vary according to the skill and experience of the surgeon. There would be no anesthetic recovery time if a general anesthetic is not used. Recovery from the tranquilizer will vary with the type and amount administered. Healing time would be approximately two (2) weeks.

2. Analysis

The following analyzes the positive and negative aspects of sterilization:

a. Positive

(1) Long-term effectiveness in mares. Although not shown to provide long-lasting effectiveness when targeted at stallions, sterilization of mares would provide permanent reproductive inhibition in those animals treated.

(2) Treatment would not require repeating on individual animals. The sterilization procedure is permanent. The process would require addressing new recruits to the population every three years for mares and more frequently for stallions. Once treated, subject animals would not require follow-up treatments.

b. Negative

(1) Non-reversible. The sterilization process is not reversible. This limits management flexibility in dealing with emergency situations.

(2) Invasive surgery. The procedure would require that the animals undergo surgical procedures. This would necessitate extensive field operations or the transport of animals to special facilities.

(3) Labor intensive. The procedures will require additional personnel including veterinarians. More time would be required to feed and care for treated animals.

(4) Increased handling of animals. The procedure would require that the animals be handled more both during the operations and recovery time.

(5) Increased stress. The increased handling and surgical operation would increase stress on the animals above what is normal during gather operations.

(6) Increased death loss. Normal handling of animals during gather operations results in some death loss (usually less than 1%). The increased handling, surgical procedures, and recovery time will increase the potential for death loss.

(7) Increased equipment requirements. The procedure will require increased equipment for conducting the surgery and additional holding pens for the recovery period.

(8) Holding time will be increased. The procedure will require that the animals be held longer until the operations are completed and recovery is ensured.

(9) Increased medical waste. The operations will result in medical waste which will require proper disposal. Gathers conducted in remote portions of Nevada will

not have immediate access to medical facilities where the waste may be properly disposed of, necessitating prescriptive measures for storage until proper disposal can be ensured.

(10) Impacts to population dynamics. The effect of sterilizing large percentages of the wild horse population could have a detrimental affect on the social structure of the herd. Band formation and integrity may be effected. There exists a lack of knowledge of long term health effects of loss of ovarian hormonal secretion in mares.

(11) Negative image. The use of sterilization has received considerable scrutiny due to the process being non-reversible.

(12) High cost. Although the cost of preforming vasectomies is relatively inexpensive, spaying mares would result in higher costs. Increased costs include: having a veterinarian on site throughout the gather; additional equipment for the operation; additional feed, man hours, inoculations to prevent the spread of disease; and loss of opportunity resulting from increased time spent on the gather site.

D. HORMONAL/STEROID IMPLANTS

The University of Minnesota conducted field trials of implanted steroids to inhibit reproduction. Two implants were used with both achieving results lasting at least three years. Silastic rods impregnated with either ethinylestradiol or ethinylestradiol and progesterone were used in the field studies conducted in central Nevada. The implants are delivered to the targeted animals through surgical implanting in the peritoneal cavity (intraperitoneal). The implants control ovulation and pregnancy in the targeted mares.

The effectiveness of the implants, as shown through the University of Minnesota research, allows for accurate computer modeling. The number of mares to be targeted for implanting would be dependent on the population size and the current reproductive rates. Preliminary modeling based on using implants indicates that reproduction rates can be reduced for six to nine years.

Mares aged 5 to 9 years old would be targeted for implants. Excess animal removals would come from ages 1 to 3 or 1 to 4. Modeling shows that targeting at least 50 percent of the mares in the 5 to 9 age group would provide for a reduced rate of reproduction in the population. These figures would be modified for each population depending on modeling and desired goals.

The steroid implants are effective for at least three years. After this time, the effectiveness declines. The implants are reversible with time and would not require intervention to restore normal reproduction. It would be possible to recapture the targeted animals and surgically remove the implants should the need arise; however, this procedure would be costly and logistically difficult.

Concern over the use of a synthetic estrogen (ethinylestradiol) has centered around the effects of its introduction into the food chain. The synthetic drug does not decompose as rapidly as natural estrogen. These animals might be eaten by carnivores whose reproduction could be impaired. Although precautions could be taken to ensure that the implanted animals never leave the range, the consumption of these animals by carnivores presents a particular problem. Natural and unnatural (poaching, vehicle strikes, etc.) mortality would invariably introduce some of the animals into the food chain. Although the National Academy of Sciences reported low risk associated with the consumption of implanted mare flesh, it was also recommended that study animals implanted with the steroids be confined to pens or sanctuaries and not be released into the adoption program or left on the range.

Besides inhibiting reproduction, steroid implants are known to affect the animal in other ways. Use of steroids also changes behavior and body conformation of the animal. Mares will become more muscular and take on stallion-like characteristics (i.e., large swollen necks).

1. Procedures

The use of silastic impregnated implants for the control of conception in the mare requires those implementing the procedure to address significant technical considerations. This procedure appears to be most appropriate in small bands of horses.

a. Restraint

(1) Squeeze chute

(a) Manual operation with side delivery for use with anesthetized mares.

(b) Hydraulic operation with or without side delivery for use with anesthetized mares or standing procedure.

(2) Chemical Restraint

Recumbent-Succinylcholine (Socostrin IM)

(a) Can be hazardous to personnel.

(b) Requires experienced personnel with access to resuscitation equipment (veterinarian).

(c) Its limited duration of action requires the operator to hasten operating time and increases risk of failure.

(3) Surgical Procedure - 10 to 15 minutes

(a) Preparation (preoperative)

1 Clipping (Surgical).
2 Skin preparation.
3 Local anesthetic.

(b) Surgery

1 Requires standard minor surgical sterile setup.
2 Trocarization success appears to be dependent on length of trocar.
3 Wound closure. Requires heavy slow absorbing suture that prevents wound dehiscence and contamination.

NOTE: Catgut suture is universally agreed to be unacceptable for skin closure. It is subject to desiccation and breakage allowing wound dehiscence and contamination.

(c) Post Operative Procedure, Wound management

1 50% Betadine solution prior to closing.
2 Antibiotics - Benzathine Penicillin.

(4) Monitoring

(a) Minimum standards for one week appear insufficient to control postoperative complications.

(b) Minimum standards for more than one week appear more suitable to control postoperative complications.

2. Analysis

The following analyzes the positive and negative aspects of steroid implants:

a. Positive

(1) Long-term effectiveness in mares. Research has indicated that implants in mares may provide from three to five years of reproductive inhibition.

(2) Reversible over time. The treatment is easily reversible with discontinuation of the treatments; the effects of the implants wear off in three to five years. The animals would not have to be handled again to reverse the process.

b. Negative

(1) Invasive surgery. The procedure would require that the animals undergo surgical implants. This would necessitate extensive field operations or the transport of animals to special facilities.

(2) Labor intensive. The procedures will require additional personnel including veterinarians. More time and money would be required to feed and care for treated animals.

(3) Increased handling of animals. The procedure would require that the animals be handled more both during the operations and recovery time.

(4) Increased stress. The increased handling and surgical operation would increase stress on the animals above what is normal during gather opera tions.

(5) Increased death loss. Normal handling of animals during gather operations results in some death loss (usually less than 1%). The increased handling, surgical procedures, and recovery time will increase the potential for death loss.

(6) Increased equipment requirements. The procedure will require increased equipment for conducting the surgery and additional holding pens for the recovery period.

(7) Introduction of chemicals to the environment. The synthetic drug ethinylestradiol, can neither be confirmed nor discounted as a threat to the environment. Scavengers that may feed on wild horse carcasses could have inhibited reproduction.

(8) Holding time will be increased. The procedure will require that the animals be held longer until the operations are completed and recovery is ensured.

(9) Increased medical waste. The operations will result in medical waste which will require proper disposal. Gathers conducted in remote portions of Nevada will not have immediate access to medical facilities where the waste may be properly disposed of, necessitating prescriptive measures for storage until proper disposal can be ensured.

(10) Impacts to population dynamics. The effect of inhibited ovulation could have a detrimental affect on the social structure of the herd. Increased hormonal imbalances could create negative long term health problems.

(11) Negative image. The University of Minnesota study generated a considerable amount of negative public opinion. The majority of the problems associated with the study were in regard to monitoring equipment and practices. Concern over introducing the steroid to the environment also generated concern.

(12) High cost. Increased costs include having a veterinarian on site throughout the gather; additional equipment for the operation; additional feed; man hours, and inoculations to prevent the spread of disease; and loss of opportunity resulting from increased time spent on the gather site.

E. IMMUNOCONTRACEPTION

Immunocontraception represents one of the most recent advances in fertility control methodology. The general principle is that antibodies are raised in the target animal against some structure or functional protein involved in the reproductive process. The antibodies hinder the fertilization process by preventing the sperm from binding with the egg. Successful immunocontraception has been achieved in both sexes. The most successful and studied method has been raising antibodies against ovarian zona pellucida.

Pen and field studies have been conducted on wild horses using porcine zona pellucida (PZP). PZP immunocontraception in wild horse mares was found to be successful in reducing pregnancy. However, the process requires that the initial inoculations be repeated with a booster shot 14-21 days following initial treatment. The contraceptive effectiveness only lasted one season. Studies by Turner and Kirkpatrick are underway to develop a one shot vaccination which will have an effective life of two or more years.

Field studies on wild horses have shown over 90 percent success in preventing pregnancy. Population models indicate that the current one year duration of control would be ineffective in the long-term management of wild horse populations. Speculative modeling with a three year effective life indicates that immunocontraception targeted at 5 to 9 year old mares would be feasible in controlling reproduction.

PZP has been shown to be reversible in only a short time frame. No side effects or environmental hazards have been identified. Some animals have experienced allergic reactions to the agent during administration of the booster; however, these cases have been extremely rare.

1. Procedures
    a. Immunocontraceptive Inoculation - Procedural Steps:

        (1) Enter squeeze chute.

        (2) Determine and record signalment characteristics, including age, sex, color, special markings, identifying marks, etc.

        (3) Observe for and record any signs of health problems, conformation defects, etc.

        (4) Determine if suitable for treatment or control group, i.e., mare, 4-9 years, healthy.

        (5) Place identifying mark on mares. Need mark that will serve two functions: 1) identify each individual animal, recognizable at least upon recapture if not from distance, and 2) identify treatment group or mare and be identifiable from long range observation (field or airplane). Freeze brand probably best.

        (6) Draw blood from neck vein into 2-3 red top tubes per horse. Identify blood samples with labels indicating individual horse identity and which treatment group she is in. Store blood for later analysis. (Refrigeration not necessary. Samples should be centrifuged and serum drawn off and saved within 48 hours after taking sample. Could be done on site with mobile lab or after return to lab facility.)

        (7) Inject bolus of long acting penicillin (Benzathine Penicillin) at 25 cc per horse. Administer deep intramuscular into rear leg hamstring muscle with needle and syringe or jab pole syringe mechanism. (Neck muscle should not be used.)

        (8) Inject PZP vaccine into mares selected as the treatment group. (At least 100 mares.) Administer deep intramuscular in opposite rear leg hamstring muscle. Direct needle and syringe, jab pole, or pellet gun (when available) could be used.

        (9) Inject sterile saline of equal volume to the vaccine into mares selected to be control group, using same equipment and technique as that in treatment group. (At least 25% of number of total mares treated, i.e., every fourth mare.)

(10) Inject other vaccines, if that is standard practice, or if planning to hold longer than 24-48 hours (VEWT, Flu, Rhino). As combining vaccines could create antigenic competition within the immune system, additional observation of the animals will be required.

(11) Release into holding pen where recapture into chute would be possible if necessary. Observe for 30 minutes for allergic reactions to inoculations.

(12) Mares could be released into herd or range within one hour of inoculations in chute. Incidence of injection site abscess should be very low and not necessitate holding for 3-4 days to observe. Complications from prolonged confinement would be higher than the expected complication rate from abscess formation.

b. Immunocontraceptive Inoculations - Equipment Requirements:

(1) Marking equipment to be decided based on monitoring plan (freeze branding probably best).

(2) 18 g, 1 inch disposable, hypodermic needles, 2-3 per horse (not to be reused).

(3) 1-20 cc syringe and 1-60 cc syringe per horse (possible reuse).

(4) Simple cleaning equipment (brushes, etc.) to remove gross contamination for injection sites.

(5) PZP vaccine requires being frozen until the day of us, and then it must be refrigerated It should not be refrozen.

(6) Injection jab poles could be helpful depending on chute arrangement.

(7) Long acting penicillin - Benzathine Penicillin - 50-70 cc per horse. (Needs refrigeration until within 48 hours of administration and should not be exposed to extreme heat.)

(8) PZP vaccine - 1 per horse, with 10-20% extra supply.

(9) Other vaccines as warranted by standard procedures.

(10) Squeeze chute and temporary holding facility - necessary only for 30-60 minutes post injection.

(11) Standard emergency veterinary medical supplies need to be on hand in case of need, including additional antibiotics, medications for shock and allergic anaphylactic reactions, supplies for treating wounds and other traumas, short acting tranquilizer or anesthesia drugs, and euthanasia solution. Routine mobile veterinary unit should be sufficient.

(12) Portable lab would be helpful, but not absolutely necessary.

c. Immunocontraceptive Inoculations - Personnel Requirements:

(1) Horse handlers as needed.

(2) One veterinarian on site as supervisor and in case of emergency need.

(3) One or two assistants, thoroughly instructed by veterinarian in administering agents properly. Licensed veterinarian technician would be advisable, but not required.

(4) One BLM supervisor to oversee all treatment procedures to assure standard technique, proper protocol and safety.

(5) Consideration might be given to having volunteers or BLM personnel with simple video cameras to record all activities to verify humane treatment and application of procedures.

2. Analysis

The following analyzes the positive and negative aspects of immunocontraception:

a. Positive

(1) Low additional cost. There would be no additional costs associated with specialized field equipment.

(2) Little additional animal handling. The capture process already requires the aging and sexing of captured animals at the trap site. Injections would be conducted during this process.

(3) Little increase in animal stress. The current capture procedures would be followed and the animals injected with the agent.

(4) Reversible and self-correcting. The treatments are easily reversible through natural reproduction and discontinuation of the treatments. The animals would not need to be captured or handled to reverse the process.

(5) No increased time requirement. The animals would not have to be held for observation following treatment. The animals would only be held until completion of the gather. This would reduce treatment cost and animal stress.

b. Negative

(1) Short term. For the treatment to be successful, it currently must be repeated every year with two injections spaced 14-21 days apart. Future developments may improve this situation.

(2) Increased medical waste. The operations will result in medical waste which will require proper disposal. Gathers conducted in remote portions of Nevada will not have immediate access to medical facilities where the waste may be properly disposed of, necessitating prescriptive measures for storage until proper disposal can be ensured.

(3) Impacts to population dynamics. The effect of immunocontraception on large percentages of wild horse populations could have a detrimental affect on the social structure of the herd. Band formation and integrity may be affected and contrary reproductive roles may be induced.

(4) Increased specialized labor. Inoculations would be given by a veterinarian or a trained technician. This will increase costs.

(5) Increased equipment. Storage of the inoculant will require special handling.

(6) Current technology requires that a booster shot be administered 14-21 days after the initial inoculations. This requires holding the animals for an extended period of time and exposing them to communicable diseases

IV. RECOMMENDATIONS

Each alternative has its own merits and limitations. These merits may make each alternative ideal in certain situations and less applicable in others. The committee was in agreement that the recommended alternatives are based on available technology and that no alternatives should be excluded from future consideration. As technology improves, other alternatives may become more desirable. The committee's recommendation is divided into two categories. The first category is General Recommendations which would be applicable for future management actions. The second category is Specific Recommendations which are particular to research/management studies which will be conducted in the Antelope/Antelope Valley Herd Management Areas.

A. GENERAL RECOMMENDATIONS

1. Selective Removals

   a. Selective removals targeting age or sex specific attributes should be considered as a standard management action for all gathers.

   b. Combining age and sex specific removals is not recommended.

   c. The age classes targeted in age specific removals should be determined through population modelling and not be limited solely to adoptability criteria.

   d. Selective removals may be used as a singular technique or in conjunction with anti-fertility drugs in fertility control management.

2. Fertility Control Practices

   a. Utilize immunocontraception (PZP vaccine) for implementation in a pilot project and for initial management considerations.

   b. Continue to review literature and research alternative forms of immunocontraception.

   c. Fertility control practices which do not require surgical procedures are preferred. Fertility control practices which require surgical procedures should be considered if they can be implemented humanely and cost effectively.

   d. Review and reconsider sterilization and implant alternatives as technology improves or if technology would provide for non-surgical procedures.

   e. Mark animals treated with anti-fertility agents to facilitate following them throughout their life cycle.

   f. Review these recommendations on a yearly basis and determine if revisions are required. Revisions would be prompted by changes in technology.

3. Combine both selective removals and immunocontraception as a management strategy.

B. SPECIFIC RECOMMENDATIONS

1. Conduct selective removals based on either age or sex specific criteria.

   a. Do not mix both age and sex specific selective removals.

   b. The combination of both types of selective removal would confound research analysis.

2. Utilize immunocontraception for the pilot project research/management study.
   a. If one-shot vaccine is available, conduct study on an HMA-wide basis.

   b. If one-shot vaccine is not available, conduct research on a portion of the population. Base the portion to be treated on research requirements and ability to safely hold animals for 14-21 days.

   c. Utilize control animals injected with saline solution and located within the same areas as those animals treated with anti-fertility antigens.

   d. When possible, conduct necropsy on all animals which have received anti-fertility treatments or control treatments and die during the study.

   e. The committee recommends that additional funding be provided to perpetuate the proposed pilot project for two additional years and expand the study scope to include additional treatment locations.

V. GROUP TWO SUB-TASK DESCRIPTION: PROCEDURES FOR MONITORING, EVALUATION AND TRACKING

Develop procedures for monitoring, evaluation and tracking of selective removals and fertility control projects.

VI. DISCUSSION

The task group identified that well defined guidelines and procedures would need to be developed for field use in implementing any fertility control actions. In discussing these guidelines and procedures, certain issues and/or constraints were identified: The following are constraints: safe and humane treatment of the animals; minimal handling; providing the type of observation which reduces animal stress; and minimizing permanent long lasting negative impacts from fertility control, monitoring and tracking guidelines and procedures. Additional issues and/or constraints are as follows:

A. Consider interactive HMAs as one for monitoring, tracking and evaluation.

B. Provide for placement of the treated animals back into the HMAs, with minimum impact to herd integrity (animals must be released at the same area from which they were removed).

C. Monitoring animal condition for possible infection of other animals, upon return to the HMAs.

D. Provide for security for the animals within the HMAs after treatment.

E. Availability of money and equipment is needed after treatment for follow up monitoring, tracking and evaluation.

F. Existing Military Operations Areas (MOAs) and restricted airspace will constrain aerial observations.

G. The ability is needed to mark treated animals for long term identification, while minimizing stress from handling.

H. Inclement weather can have an effect on follow up actions.

I. Marked animals need to be visible from the ground and the air.

J. Aerial and ground observations during the foaling season may cause additional stress to the animals.

K. Habitat condition can have an effect on the results of the fertility control project (i.e., drought, ecological status).

L. Selective removals (skewing age distribution) may have effects on the results of other fertility treatments.

These issues and/or constraints must be taken into consideration when selecting the chosen procedures for monitoring, tracking and evaluation of the fertility control projects. This would then allow evaluation of the results for future applications.

VII. OPTIONS/ANALYSIS

This task is broken down into three parts: monitoring, tracking and evaluation. Monitoring will evaluate habitat condition, the condition of the animal upon release back to the public lands, and continued monitoring of the animal for the effects of the fertility treatment, with the primary purpose of determining changes from baseline data in animals and herds alike. Tracking will provide the ability to relocate marked, treated animals for identification at a later date while monitoring. Evaluation will compare the results from the fertility study to each individual HMA's baseline data.

To adequately conduct an evaluation at the end of the fertility studies, sufficient data must be collected and/or documented as baseline data. This baseline data can then be compared to subsequent data collected after the implementation of the study. Therefore, the following information must be collected and/or documented as each HMA's baseline data for future reference.

Required HMA Baseline Data (standard in Nevada):

A. Condition of animals.
   1. Physical condition.
      (i.e., weight, fleshiness, coat, stress)

   2. Behavior.

   3. Disease.

B. Band structure.
   1. Size, composition, distribution and number of bands.

   2. Historical band characteristics.

C. Age structure at time of capture (adults to foals).

D. Recruitment rates.

E. Seasonal movement patterns and interaction between HMAs.

F. Grazing habits.

G. Habitat condition.

H. Total number of animals in population.

I. Additional data and/or studies available or determined required for each individual HMA (i.e., ingress/egress; where possible band intermixing; genetic studies; social behavior; etc.).

The following are the proposed methodologies for monitoring, tracking and evaluation of population control alternatives. Activity plans must be developed or amended to incorporate these methodologies.

SELECTIVE REMOVALS, FERTILITY CONTROL TREATMENTS
(sterilization, steroid implants, immunocontraception)

A. Monitoring.

1. Short-term monitoring for condition of animals upon release.

Minimum standards will be to monitor a horse's condition by ground and/or air within 24 hours of their release. Aerial observation should be scheduled for no later than 72 hours after release to assure no animals are trapped on a fence or other obstacle, or are without access to water. If needed, ground checks following the aerial observations should be used. The collection of this data will be used for a comparison to the baseline data elements for the HMA.

Each district must conduct advance coordination with the military for activities within Military Operations Areas (MOAs). Planned aerial flights will resume as soon as possible after aviation restrictions are revoked by the military. Sufficient ground observations must be completed to substitute for the lack of aerial observations during restrictions.

2. Long-term Monitoring for effects of the population control on the animals.

a. Aerial and ground observation will be conducted on a yearlong basis to collect monitoring data related to the baseline data elements. This may include a census flight after each foaling season to collect monitoring data on recruitment rates, age structure (adult-yearling-foal), seasonal movement patterns, and grazing habits. Foal count after the foaling season and then again before the next foaling season will provide foal survival information.

b. Collect those additional studies determined to be necessary for each individual HMA (i.e., ingress/egress; where possible band intermixing; genetic study; social behavior).

c. The pilot project will establish a monitoring program which will be dictated by the specific objectives of the research and the project duration. At a minimum, long-term monitoring will follow those criteria stated above.

B. Tracking

1. Temporary marking.

Animals held and removed from the trap site must be marked to assure their return back to the capture site. This will be done through temporary marking using grease pencil, paint, etc. Type of marking would depend on their length of stay in a holding facility.

2. Permanent marking.

a. Visible from the field.

(1) Freezemark. Marking must be large and placed on the animal where readily visible (i.e., hip). The symbols and location used for the freezemark should tie to specific information required for evaluating each group of treated animals. Information provided in the freezemark should include age, sex, treatment, herd area or other information pertinent to a specific treatment group. The specific freezemark used will be determined for the treatment, the type of data to be represented by the mark, the duration of the study, and proximity to other study areas. The freezemarks used will be specific to each study group to avoid confusion from potential mixing from other study areas. Figure 1 identifies potential freezemark applications.

(2) Collars. Readily visible, but need to be fitted properly (neither too snug nor too loose).

(3) Telemetry. These could be implanted using microchips or using a properly fitted collar.

b. Visible only by recapture. Freezemark. Marking must be smaller and/or placed on the animal (i.e., neck) where easily viewable only upon recapture. The symbols and location used for the freezemark should tie to specific information required for evaluating each group of treated animals. Information provided in the freezemark should include age, sex, treatment, herd area or other information individual to a specific treatment group.

c. Combination of the above.

FIGURE 1
Potential freezebrand marks for fertility control study animals.

Freezemarking

All treated animals will be branded high on their left hip and all control animals will be branded high on their right hip. The following are examples of the freezemarks for each potential treatment:

Immuno-contraceptive* (female)

U.S.	Age
	91

Hormone Implant (female)

U.S.	Age	Treatment
	91	H

Sterilization (female)

U.S.	Age	Treatment
	91	S

Sterilization (male)**

U.S.	Age	Treatment
	91	

*Animals treated with immuno-contraceptives will be branded with only the U.S. ownership symbol and their date of birth.
**Assumes that only sterilization will be used on males.

C. Evaluation

1. General. The evaluation of each population control project must be designed to determine the success or failure of the project. Therefore, the success parameters or objectives to be accomplished must be defined initially within the activity plan (i.e., limit the recruitment rate within the Antelope/Antelope Valley HMAs to < 10% within the next two years). There are three primary parts of the evaluation.

   a. Evaluate the selective removal/fertility control results. Compare the HMA population dynamics (i.e., age structure, foaling rates, recruitment rates, death loss, etc.) before and after implementation.

   b. Evaluate the effects of the capture and release/fertility control on the animal's condition, general health, behavioral patterns, and social structures.

   c. Evaluate the effects of environmental conditions upon the selective removal/fertility control results (i.e., drought, habitat condition, diet, etc.).

2. Standardization. All individuals involved in the project should understand methodology of not only data collection, but analysis. Applied methodology must be consistent throughout the project (i.e., actual census vs. modeling). This should be standardized for future use by each district. When available, manuals, regulations and existing guidance should be followed.

3. Selective removal/fertility control results.
   a. Minimum baseline data to be collected should include: census recruitment (rate of increase), age structure, foaling rates, and death loss.

   b. Comparison of baseline data to post removal information.
      (1) Foaling rate (if possible).
      (2) Pre- and post-removal recruitment (rate of increase) comparison.
      (3) Other information as required germane to a specific treatment group.

4. Effect of selective removal/fertility control on animals.

   a. Minimum baseline data to be collected should include: animal condition, band structure, grazing habits, and seasonal movement patterns.

   b. Comparison of baseline data to post removal information.
      (1) Physical - weight gain or loss, lethargy, illness, etc.
      (2) Social - band structure, movement, relationship of mares to others in and out of bands.
      (3) Behavioral Patterns - grazing habits, seasonal movement patterns, etc.

5. Effects of environmental conditions on selective removal/fertility control results.

   a. Minimum baseline data to be collected should include: habitat condition, utilization levels, precipitation, water availability, and diet.

   b. Determine if environmental factors affected the selective removal/fertility control results. Compare baseline vegetative monitoring studies (i.e., utilization levels, precipitation data, actual use and ecological status) to post-implementation.

6. Success. Success will be measured against specific objectives defined within each activity plan. Objectives must be measurable, realistic, and attainable within established timeframes (i.e., recruitment rate and animal condition).

VIII. RECOMMENDATIONS

The options available for monitoring, evaluating and tracking are limited by the nature of the mission, the amount of information and level of detail desired, and the availability of funding. To ensure a quality effort in wild horse and burro fertility management, only those procedures which best fulfill the needs of each monitoring, evaluating and tracking were proposed. The committee's recommendation is to accept those procedures and guidelines as outlined in section VII. OPTIONS/ANALYSIS as presented.

IX. PERSONNEL AND TRAINING NEEDS

Implementation of a fertility control program will require animal management and monitoring practices previously not experienced within the wild horse and burro program. As fertility control represents new technology, additional knowledge and skills will be required of BLM personnel to transform research concepts into management application. Additional time required for gather/treatment operations and population monitoring of treated herds will usurp time from existing vegetation monitoring and censusing programs.

As fertility management represents new technology, training and education programs will be required for current wild horse and burro specialists. This will be coupled with recruiting additional specialists with backgrounds diverse in animals science and population management skills.

A. Personnel Requirements

The current wild horse and burro staff is comprised of professionals with training and education in wildlife management and/or rangeland management. Although these skills provide the foundation for interdisciplinary wild horse and burro management, implementation of fertility management will require additional skills. It is recommended that the BLM pursue the recruitment of individuals possessing the following skills.

1. Animal Science
   Fertility management will require a detailed knowledge of equine physiology, nutritional requirements, reproductive physiology, and diseases.

2. Technicians
   Increased handling of animals will require that personnel with skills in animals restraint, application of nominal medical treatment, aging, and field classification of animal condition.

B. Training Needs

In addition to maintaining the current skills of wild horse and burro staff professionals, additional training will be required to implement field operations associated with fertility management and to maximize scarce time resources. Training for personnel involved in the pilot fertility project will be provided in late summer 1992. Additional training on a statewide basis will be developed using knowledge acquired during initial implementation of the pilot project. Training will be required for the following subjects.

1. Animal Management
   a. Application of inoculations of vaccines and immunocontraceptives.
   b. Handling of medical equipment. This will include the storage and safety precautions of drugs and medical equipment and the safe disposal of all medical waste.
   c. Classification of animals' physical condition and identification of equine diseases.
   d. Aging of animals.
   e. Freezebranding techniques and proper handling of freezebranding equipment.
   f. Animal handling and restraint.

2. Monitoring
   a. Animal census and population dynamics monitoring techniques.
   b. Habitat monitoring and data analysis techniques. This would include use pattern mapping, ecological site inventorying, and trend study methodologies.
   3. Fertility Management Techniques

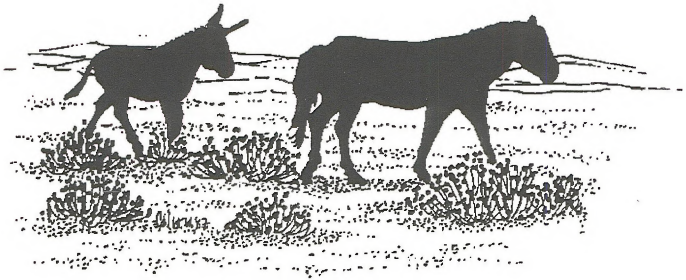
Education of the various techniques available for fertility management with emphasis on immunocontraception. Additional education would be provided regarding population modelling, herd dynamics and HMAP development using fertility control and population modelling.

X. RECOMMENDATION: ANTELOPE/ANTELOPE VALLEY HERD MANAGEMENT AREAS

The fertility control method recommended for use in the Antelope/Antelope Valley pilot project is a combination of a selective removal and immunocontraception. The selective removal will be implemented to attain appropriate management levels and will target removals at young, adoptable animals. Immunocontraception will be implemented to reduce the reproductive rate in the remaining population.

Immunocontraception will involve injecting a proportion (based on population modelling) of all mares in the HMAs with a drug that requires one shot, if available. If a one shot drug is not available, one injection will be given to a small sample of mares. These mares will be held in captivity for 2-3 weeks, will be given a booster inoculation and then will be released. A proposal for implementing the pilot fertility project is located in Appendix A.

Methodology for implementing, monitoring, tracking, and evaluation will follow all guidelines described in the state-wide recommendation.



FERAL HORSE AND BURRO FERTILITY CONTROL IN NEVADA: CONTRACEPTIVE VACCINE PILOT PROJECT

A Proposal Submitted to the
United States Department of the Interior
Bureau of Land Management
Nevada State Office

by

Kenneth W. Hunter, Jr., Sc.D.
Professor of Biology
Associate Vice President for Research
and Dean of the Graduate School
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July 1990

The University of Nevada, Reno (UNR) is pleased to submit this proposal for a pilot project to evaluate a novel, single-injection contraceptive vaccine for fertility control in feral horses. Wild horses and burros in Nevada represent a magnificent natural resourse, but a resource that requires better management. We feel that an immunologic approach to fertility control represents a humane and cost effective way to manage wild horse and burro populations on public lands in Nevada and elsewhere.

While a variety of potential population management approaches have been discussed in the scientific community, the use of a zona pellucida-based vaccine in mares is perhaps the approach with the greatest present potential. In this proposed project, UNR will subcontract with the Medical College of Ohio for the services of Dr. John W. Turner and his colleagues Drs. Jay G. Kirkpatrick and Irwin K.M. Liu, acknowledged experts in the preparation and use of zona pellucida-based vaccines for fertility control. UNR will serve an administrative role, and provide oversight on the project through the following faculty committee:

Kenneth W. Hunter, Jr., Sc. D., Professor of Biology, Associate Vice President for Research and Dean of the Graduate School (Committee Chair)

Donald R. Hanks, D.V.M., Professor and Chair, School of Veterinary Medicine

Richard C. Simmonds, D.V.M., M.S., Director, Laboratory Animal Medicine

William G. Kvasnicka, D.V.M., Associate Professor of Veterinary Medicine and Extension Veterinarian

Ronald S. Pardini, Ph. D., Professor of Biochemistry and Associate Director, Nevada Agricultural Experiment Station

Duane L. Garner, Ph. D., Professor of Animal Science

This committee will meet periodically with the research team from the Medical College of Ohio and the Nevada Bureau of Land Management to plan and discuss the progress of the pilot fertility project.

The following section of this proposal outlines the experimental approach for the pilot fertility control project.

INTRODUCTION

Feral horse management on western public lands is currently confined to the removal of excess horses. While we are not convinced that there is an actual overpopulation of horses in many areas, we recognize the need for improved, more effective management of feral horse populations. The removal of horses as the sole management effort, while seemingly effective at the time of removal, does not prevent the subsequent growth of the remaining population and insures that removal must continue year after year. Indeed, there is evidence that the removal of horses actually increases fecundity among those animals remaining behind and accelerates the growth of the population (Kirkpatrick and Turner 1991). In other words, removal alone addresses only the symptom of overpopulation (too many horses) and not the cause (reproduction).

An alternative approach is to limit reproduction, through some form of fertility control (see reviews by Kirkpatrick and Turner 1985, 1991; Turner and Kirkpatrick 1991). Toward that goal we have tested a contraceptive vaccine on feral horses which can limit the number of foals born to free-roaming mares. The major characteristics of this vaccine include (1) great effectiveness (> 95% effective), (2) remote delivery, which permits humane non-capture administration of the vaccine, (3) relative low cost, (4) no effects upon individual or social behavior of the target animals, (5) no effects upon pregnancies already in progress at the time of delivery, (6) reversible contraceptive action, and (7) no passage of the vaccine through the food chain or into the environment. These characteristics have been previously identified as required for successful feral horse contraception (Turner and Kirkpatrick 1986).

The vaccine, known as porcine (pig) zonae pellucidae, or PZP, satisfies these criteria. The zona pellucida is a non-cellular protein membrane which surrounds all mammalian eggs. In order for fertilization to occur, sperm must first bind to this membrane before they can penetrate the egg. The intramuscular injection of PZP into mares causes them to produce antibodies against the pig protein, but these antibodies also bind to the sperm attachment sites on the mares' eggs, thereby preventing sperm attachment and fertilization (for a review of the PZP vaccine see Paterson and Aitken 1990). Because only fertilization has been blocked, there are no hormonal manipulations which cause behavioral changes. Indeed, immunized mares remain together in their social groups, ovulate regularly during the breeding season, and permit mating behavior by the herd stallion, and in general reflect the social behavior of untreated feral horses (Kirkpatrick et al. 1990a).

This vaccine was originally tested on captive feral horses and prevented pregnancies in 13 of 14 treated mares (Liu et al. 1989). Following this, the vaccine was tested on free-roaming feral horses managed by the National Park Service (Kirkpatrick et al. 1990a).

The hallmarks of this first field test were successful remote delivery by means of barbless darts fired from a capture gun, a demonstration of the vaccine's effectiveness (no pregnancies among 26 treated mares vs. a 50% pregnancy rate among control mares), reversibility, and a demonstration of its safety for use in animals already pregnant at the time of inoculation. After four years of treatment over 60 "mare years" (i.e., the number of mares treated annually x the number of years treated) only a single foal has been born. This approach to fertility control in feral horses has been so effective that the National Park Service is already in the process of designing a management program built around this vaccine (personal communication, John Karish, Regional Scientist, Mid-Atlantic region, National Park Service). The effectiveness and safety of this contraceptive vaccine has been well documented and our own research group has tested the vaccine on a variety of other hoofstock, including white-tailed deer (Turner et al. 1992), sika, samabar, axis and muntjac deer and Himalayan tahr (Bronx Zoo), and West Caucasian tur (Toronto Zoo). Other investigators have demonstrated the effectiveness of the vaccine in a wide variety of non-human primates (Paterson and Aitken 1990) and even humans (Sacco 1987). Currently the vaccine is a candidate for development as a human contraceptive (Millar et al. 1989).

The vaccine has one major disadvantage at the present time. During the first year of administration of the vaccine, the mare must be inoculated twice, about three weeks apart. Contraceptive protection for subsequent years requires only a single booster inoculation (Kirkpatrick et al. 1992). Thus, the focus of current research efforts is to develop a one-inoculation vaccine which will permit one to two full years of contraception after a single administration. Basically, this will involve incorporating multiple doses of the PZP vaccine in a single inoculation in such a way that there is an initial release of some of the vaccine after injection and then a small but constant release of the remaining vaccine, similar to the way Contac® cold capsules work. A pilot study has already been carried out which has demonstrated the effectiveness of a continual release of the vaccine. This study, with domestic mares, employed a single injection followed by placement of an implant under the skin, which released the vaccine gradually over four weeks. Antibodies were produced in quantities which cause contraception and indicate that a one-inoculation sustained release system can be effective as a fertility inhibitor (see Figure 1).

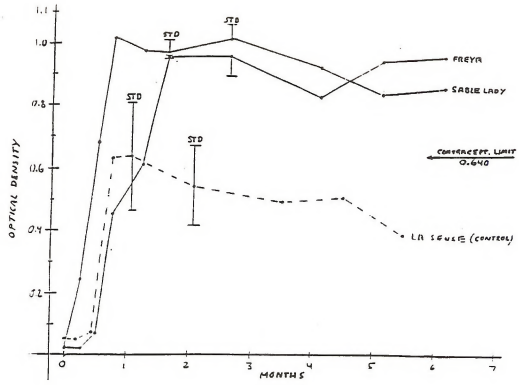

Fig. 1. Effect of sustained-release PZP vaccination in mares (Freya and Sable Lady) on anti-PZP antibody production. Vaccination consisted of bolus injection of 65 µg PZP and Freunds Complete Adjuvant (0.5 cc) followed by sustained PZP release (2.3 µg/day) over 28 days from a subcutaneously implanted osmotic minipump (Alzet, Inc.). Control (LaSense) received bolus PZP and Freund's Complete Adjuvant, but no pump. Plasma antibody titers are measured in optical density units. The lower limit of contraceptive efficiency determined from previous studies is 0.64 O.D.

With these encouraging data we have embarked upon the chemical engineering necessary to give us this same type of release pattern in a single injection. This is a collaborative effort between the Medical College of Ohio, Deaconess Research Institute, the University of California at Davis, and The Humane Society of the U.S.. The prototype timed-release preparation is already underway and we expect to have an initial testing of it in domestic mares completed by Fall of 1992. Additional funds are needed to complete this study, and this is the first of three studies for which we are requesting funding from your organization.

The second study for which we are requesting funding support is the development of a two-year contraceptive capability with a single injection. This will essentially involve an extension of the technology for the annual single-injection vaccine described above. It is obviously more time- and cost-efficient to deliver vaccine every other year instead of annually. The timed-release technology which is currently available must be evaluated for its specific application to the PZP vaccine. This approach involves formulating a single injection which contains the two-dose release sequence for the first year and a single dose released 9-12 months later for contraception during the second year. Long-term timed-release such as this, employing a process called microencapsulation, has been used for other applications (Eldridge et al. 1989). The high potency of the vaccine in small amounts makes it a very good candidate for permitting microencapsulation and still allowing remote delivery.

While the two studies described above will be primarily chemical engineering (with testing of antibody levels in domestic mares), the true test of the vaccine will require a field study. To accomplish this, the vaccine will be tested on free-roaming feral horses in Nevada. This third study, for which we are requesting funding, will be carried out in one or two herd areas mutually agreed upon by our research group and the agency or agencies appointed to make such decisions in Nevada. The field trials will evaluate effectiveness of the vaccine by pregnancy testing and foal counts. While remote delivery of vaccine in the field by darting from helicopter or at water holes is certainly a reasonable eventual goal, the proposed field trial will focus on injection in the chute following gathering. This will permit guarantee of scientific validity in terms of assured injection of vaccine and individual animal identification. Other field trial considerations such as cost, time, humaneness and safety will be monitored. While it is possible that the chemical engineering of the single-injection vaccine will be completed by Fall of 1992, we cannot guarantee this. Therefore, we propose two possible vaccination protocols for the 1992 gathering. If the single-shot vaccine is complete at that time, one half of the mares will be given a single injection and released while the remainder will be injected, retained for 3 weeks, reinjected and released. If the single-shot vaccine is not complete, then our current 2-injection procedure will be used on all mares. The proposed protocol will require maintaining horses in captivity for 3 weeks (without handling), but will permit successful vaccination and maintained flow of the project in the event that the single-injection engineering is delayed in completion. Because the second study (i.e., two-year capability) will probably not be complete by the time the initial field applications are needed the proposed first round of field testing will utilize only the prototype annual single-injection vaccine or current two-injection procedure.

While this proposal is brief and to the point, it is important in outlining crucial steps to enable large scale contraceptive vaccination of feral horses. We feel it is necessary to point out that the alternative available contraceptive technology - steroid

hormone implants - does not represent current technology nor does it satisfy basic criteria for humane treatment of animals. It is not cost-effective, safety for use in pregnant animals is still a question, behavioral effects are unknown, and steroid use is not likely to be permitted by the EPA because of possible environmental and food-chain contamination.

RESEARCH PLAN

Rationale

The purpose of this proposed research is three-fold and includes (1) development of a functional one-inoculation, one-year PZP contraceptive vaccine which can be delivered remotely for the regulation of free-roaming feral horses, (2) extension of that engineering technology to produce a one-inoculation PZP vaccine which will provide two-years of contraceptive protection, and (3) field test of the vaccine on free-roaming feral horses inhabiting public lands in Nevada.

Objectives

The specific objectives of this proposed research include the following:

I. Development of the one-inoculation, one-year vaccine (in the form of MICROSPHERES).

   1. to determine if the PZP protein, or antigen, retains immunological activity during preparation for incorporation into microspheres,

   2. to engineer a sustained-release formulation for a one-inoculation PZP vaccine that will impart a full year of contraceptive protection, i.e., microspheres,

   3. to test the effectiveness of this one-inoculation, one-year vaccine to produce antibodies in domestic horses.

II. Development of a one-inoculation PZP vaccine which imparts two years of contraceptive protection (in the form of MICROCAPSULES).

   1. to determine whether the PZP antigen retains immunological activity during preparation for incorporation into microcapsules,

   2. to engineer a timed-release, pulsed-release formulation for a one-inoculation vaccine which will impart two-years of contraception,

   3. to test the effectiveness of the one inoculation, multiple year PZP vaccine to produce antibodies in domestic horses.

III. Remote field testing of the PZP vaccine in its current 2-injection form or as a single-injection prototype on free-roaming horses in Nevada. Note that additional field trials will be needed to complete PZP vaccine testing, and these will be addressed in a subsequent proposal.

Considerations in the development of a one-inoculation PZP vaccine

At the present time a minimum of two inoculations of the PZP vaccine, given three weeks apart, are necessary for effective contraception in horses. Despite the > 95% contraceptive effectiveness of the vaccine, the need for two inoculations greatly limits the usefulness of this approach for use in free-roaming horses. Thus, the first goal of this proposed research is to develop a method for delivering a single inoculation of PZP vaccine which will result in an immediate release of some of the vaccine antigen, and then a second release of the vaccine, either continuously for a month or so or as a pulsed release about 3 weeks later. Ideally, the one inoculation would also contain a third dose of the vaccine which would be released about one year later, thus resulting in contraceptive protection for two or more years.

There are two existing technologies which can immediately be applied to the PZP vaccine to meet these goals. The first is to bind the PZP antigen within an inert non-toxic polymer which, upon injection, will release the antigen continuously but slowly over some period of time. The chemical particles which contain the antigen are referred to as microspheres. The second technology is microencapsulation of the PZP antigen. This involves coating the antigen with a non-toxic material which, after injection, erodes away and also releases the antigen. Microcapsules differ from microspheres in that they cause a sharp, timed, pulsed release of the antigen rather than a sustained release (Maulding 1987).

The first timed-release approach involves the continuous, controlled release of PZP antigen imbedded within a microsphere matrix of poly (L-lactide) or copolymers of lactide and glycolide. This approach has been used for the delivery of a large number of drugs, including intramuscular and subdermal contraceptive agents, cancer chemotherapeutics and vaccines (Cowsar et al. 1985; Linhardt 1989; Staas et al. 1991). This methodology initially appeared less promising than microencapsulation (see below) because the process causes a continuous release of the antigen rather than pulses, and continuous release might result in tolerance to the antigen rather than production of high concentrations (titers) of antibodies. However, our preliminary study of continuous release of PZP antigen in mares (see page 4, Figure 1) has demonstrated that high titers of antibody, well above the contraceptive threshold, can be obtained by continuous release. These results make this approach very attractive. Microsphere release of a common protein (bovine serum albumin, or BSA) indicates that this process can duplicate the release we achieved with the implant (see Figure 2). The two real critical questions are whether or not the PZP protein will withstand the chemical process required for incorporation into

microspheres and whether microsphered PZP vaccine will work in vivo.

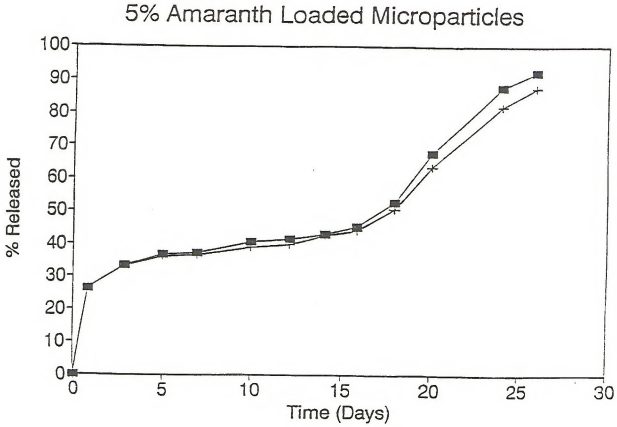

Figure 2. Release rates for bovine serum albumin from lactide pellets.

The technology to produce a one-inoculation PZP vaccine by microencapsulation also already exists. Several protein vaccines have been microencapsulated for oral delivery in humans (Eldridge et al. 1989), and there is a high probability that the same thing can be done for the intramuscular injection of PZP antigen. In the microencapsulation process the protein antigen, PZP in this case, is coated with a non-toxic polymer material, producing small capsules about the size of talcum powder grains. Upon injection into the animal the coating begins to erode. When erosion is complete, the PZP is released. We have previously used this very technique - microencapsulation - to deliver contraceptive steroid hormones to feral horses (see Kirkpatrick et al. 1982; Turner and Kirkpatrick 1982; Turner and Kirkpatrick, 1991). Long-term release rates for vaccines incorporated into microcapsules have been reported to be maintained for up to 2 years (Staas et al., 1991) and we expect that the same sort of sustained release can be achieved with the PZP antigen. Once again, the two critical questions are whether the antigen can withstand the chemical process required for incorporation into microcapsules and whether

the preparation works in vivo.

There are several laboratories which can microencapsulate protein molecules. The most established microencapsulation laboratories in the U.S. are Southern Research Institute (Birmingham, AL), and Medisorb Technologies (Cincinnati, OH). Their approach is to coat the protein antigen with a non-toxic biodegradable coating (D,L-lactide and D,L-lactide co-glycolide) which, on contact with tissue fluids breaks down into harmless products such as carbon dioxide and lactic acid (Redding et al. 1988). When the coating erodes, the protein antigen is released and stimulates the animal to produce antibodies which will bind to its own zonae pellucidae, on its own eggs, and thereby block fertilization.

Considerations for field tests of the one-inoculation vaccine:

Regardless of the success of the chemical engineering necessary to develop the one-inoculation vaccine, the ultimate measure of success in this project will be the effectiveness of inhibiting fertility in PZP-treated free-roaming feral horses in Nevada. Thus, the second major component of this project is to test the one-inoculation vaccine under field conditions. This will involve selection of an appropriate herd area in Nevada, gathering of horses at the appropriate time administration of PZP vaccine or placebo to identified mares in the field and monitoring of these mares for pregnancy and foaling.

METHODS

STUDY 1

PZP Microsphere Development: This work will be performed under subcontract, in the laboratory of R. Linhardt, at the University of Iowa. Approximately 3.0 mg of PZP will be obtained from I.M.K. Liu, at the University of California, Davis. The PZP will be tested for its ability to withstand concentrating, lyophilization, organic solvent exposure, desalting, and heat exposure. These tests are necessary to determine if the PZP antigen can withstand the actual chemical processes necessary for incorporation into microspheres. Retention of the PZP's ability to raise antibodies will be determined by a procedure known as western blot electrophoresis, using PZP anti-horse antibodies already prepared at U.C.-Davis, by M. Bernoco. If the PZP retains its ability to raise antibodies, the next step is to actually incorporate 65 μg doses of PZP, along with an appropriate adjuvant, into microspheres. These microspheres will then be injected into 3 domestic horses, at the Equine Reproduction Laboratory at U.C.-Davis. Periodic blood samples will be collected to determine if the horses are raising antibodies against the microspheres.

Microsphere preparation and in vivo testing: If antibody titers sufficient for contraception are obtained, the most promising formulation will be prepared for injection into a larger number of domestic horses. Preparation will be by R. Linhardt and associates using procedures previously described (Wang et al., 1990, 1991).

PZP release rates will be designed on the basis of previously effective doses in horses, such that 65 μg is released initially and 65-90 μg is released continuously thereafter over one month. Also, Freund's Complete Adjuvant (FCA) will be used based on previous success with this adjuvant in horses. Adjuvants are compounds which, when given with a vaccine, cause the target animals' immune systems to produce very high concentrations of antibodies against the vaccine. A study is already underway which is investigating the possible use of other adjuvants which have minimal side effects and maximum antibody responses. This adjuvant study, conducted by us and funded in part by the American Association of Zoological Parks and Aquariums (AAZPA) will run parallel to our research on a one-inoculation PZP vaccine and will provide valuable information for identifying sound adjuvants for use with the PZP vaccine in horses. The expanded horse study will utilize domestic horses at the Equine Reproduction Laboratory at U.C.-Davis, and will be supervised by Dr. I.K.M. Liu.

Study Design

Group 1 - Free PZP bolus and PZP microspheres + FCA (n=5)

Group 2 - PZP microspheres + FCA (n=5)

Group 3 - Empty (or BSA-loaded) microspheres + FCA (n=5)

Study Schedule

1. Immunization injection 6 weeks prior to onset of breeding is preferred.

2. Blood sample prior to inoculation and monthly post-inoculation for antibody titer measurement.

3. Fecal and/or urine samples prior to inoculation and monthly post-inoculation to determine pregnancy. This will be performed by J. F. Kirkpatrick, Deaconess Research Institute, Billings, MT and will provide information regarding contraceptive efficacy eight months prior to expected foaling time, thereby permitting maximum lead time for designing the next phase of the research.

4. All mares will be placed with fertile stallions and the above schedule of collections and tests will be carried out until antibody titers drop below the contraceptive threshold (previously determined by I.K.M. Liu et al. (1989); all animals will be monitored for general health and physical condition during the study.

Part of Study 1 is already underway as a collaborative effort between The Humane Society of the U.S., the Medical College of Ohio, Deaconess Research Institute, the University of California at Davis, and the University of Iowa.

STUDY 2

Two-year contraceptive vaccine with a single inoculation (microencapsulation): This is primarily a chemical engineering study and will involve subcontracting with one of several companies (Southern Research Institute, Birmingham, AL; Medisorb Technologies, Inc., Cincinnati, OH) to formulate the PZP preparation according to the timed-release schedule we request. Testing of antibody-stimulation characteristics will be performed by I.K.M. Liu. Basically this research will follow the same steps described above for the one-year microsphere inoculation, i.e., (1) testing of the antigen for its ability to withstand the process of microencapsulation, (2) incorporation of PZP antigen into microcapsules designed to give a release one-month, and 10 months after injection, and (3) in vivo testing of microcapsules in domestic horses. Depending upon the start-up date, this projected research will permit in vivo testing in domestic mares by Fall of 1992.

STUDY 3

Field study of one-inoculation PZP vaccine

Selection of field site: A feral horse herd in Nevada will be identified and agreed upon for field test of the PZP vaccine. Selection will require mutual agreement by our research group, the Bureau of Land Management and the State of Nevada. Selection criteria will include (1) topography suitable for testing, (2) herd size suitable for testing, (3) available background data regarding fertility rates, mortality rates, and population dynamics which will permit reasonable population modelling, and (4) available logistical support (housing, transportation, etc.). The site presently under most serious consideration is the combined herd management areas of Antelope and Antelope Valley in eastern Nevada. All agencies with regulatory authority over the test animals must agree, in writing, that only horse gathers or removals associated with the experimental design of this study will be conducted during the course of these studies.

For the selected feral horse population several population parameters must be established before treatment can begin. First, the desired population effect must be determined. This can be stated as a question; do we wish to achieve negative growth, zero growth, or some predetermined low growth rate? Second, once the desired population effect has been decided upon, we must determine what percentage of sexually mature mares must be treated in order to achieve the population effect, i.e., 60%, 70%, etc. Finally, we suspect that there are differential fecundity rates among mares with foals (yearlings at the time of treatment) and those without foals. Recent evidence from feral horses in California (J. W. Turner, unpublished data) and on a barrier island (Kirkpatrick and Turner 1991) indicate that mares without foals are more likely to be pregnant than those with foals and are less likely to become pregnant the next year. In the herd or herds to be treated in the proposed studies contraceptive treatment efforts will include as

many mares with foals as possible. The determination of the population goals, size of the target treatment population, and which individual animals provide the best opportunity for contraceptive success are the domain of population modelling (we suggest Dr. Walt Conley, New Mexico State University, for this input), and these parameters will be assessed before actual treatment begins. As a first estimate regarding the Antelope (n=468) and Antelope Valley (n=540) HMA's, based on discussion with informed BLM personnel, an "n" of 100-140 mars in the 5-9 year age group may be available for the study. Prior to beginning the field test it must be demonstrated that the herd is in reasonably good nutritional state, 2) the range is in fair to good forage condition with reliable water availability and that adequate gathering/holding capabilities exist to carry out the study.

Treatment Procedures: Gathering by bands is preferred to insure family integrity. However, our experience has been that gathered horses which have been separated from their bands and then released back into their home range area have good probability of relocating and rejoining their original band. Gathered females will be individually identified by freeze-brand marking. Pregnancy can be determined via urine sample testing on site (Roser and Lofstedt 1989) and injection of selected mares can be accomplished by jab-stick in chutes, or blowpipes in the corrals.

PZP antigen for these field tests will be produced by I.K.M. Liu, at U.C.-Davis. The PZP-loaded microspheres and/or microcapsules will be formulated and produced by the appropriate subcontractor (Linhardt, University of Iowa; Southern Research Institute; Medisorb Technologies, Inc.). Delivery of PZP vaccine to horses will be conducted/monitored by members of our research group.

Only healthy mares (as determined by our research team veterinarian) will be used in the study. Treatment of mares will be done in a blind study initiated in fall/winter based on the successful protocol developed in the course of the Assateague Island studies. Pending availability of single-injection vaccine and 140 mares for treatment, the following groups and numbers will be included: 2-injection PZP (55), 2-injection placebo (15), 1-injection PZP 956), 1-injection placebo (15). The 2-injection groups are essential in this study as a reference base with which to compare the 1-injection preparation. As stated in the Introduction section, Introduction section, if the 1-injection prep is not available by the time the treatments must be done, all mares will be given the 2-injection protocol. This will insure a viable field trial of PZP vaccine in 1992. Observations will be made of the horses during the ensuing breeding season in order to document that social structure is intact and to determine if there is any significant change in behavior. Essentially we are interested in whether or not harem groups are intact, whether mares are being attended by the stallions, and whether mares are displaying clinical signs of behavioral estrus. Additionally, a certain number of treated mares with unique identifying markings will be photographed for later identification. This will be important for

determining the duration of contraceptive effects.

Although the initial test will utilize gathered horses and direct injection of vaccine, an important consideration for vaccine delivery in the future is remote darting. Therefore, preliminary evaluation of this issue will be undertaken in the proposed studies. Capture gun technology is designed primarily for immobilizing animals, and not for remote delivery of drugs. Modifications of equipment and techniques of delivery are required to deliver drugs remotely to free-roaming animals and our experience with feral horses on Assateague Island has provided a great deal of experience in this area. There are currently several brands and models of capture guns and self-injecting darts which can be considered candidates for this work. These include the Pax-Arms rifle, Pneu-dart, Inc., and the Teleinject system. Additionally, Dr. Lee Simmons, of the Omaha Zoo, can provide custom capture rifles. Each of these instruments has advantages and disadvantages and it is our intention, in the course of this study, to evaluate all systems and seek appropriate modifications in order to achieve the greatest success. It is important to remember that, even when the one-inoculation vaccine is available, it will do little good if we can't get it into the horses.

Pregnancy diagnosis: At the time of the gather (1992) blood/fecal samples will be collected for pregnancy testing. Mares given 2-injections of PZP will also be blood sampled at the time of 2nd injection for antibody titer testing. Between August and November (1993) following the breeding season urine and/or fecal samples will be collected from a statistically valid sample of the treated and untreated populations. The urine and fecal samples will be collected as described by Kirkpatrick et al. (1988, 1991a), and measured for pregnancy-dependent estrone conjugates and non-specific progesterone metabolites as described by Kirkpatrick et al. (1988, 1990b, 1991b). The establishment of pregnancy rates is important because foaling rates do not always provide accurate pictures of contraceptive effectiveness. Fetal loss and early foal mortality (the latter witnessed by J. W. Turner among California feral horses where foals are subject to lion predation) can confound the measurement of contraceptive effectiveness; early pregnancy determination can provide a more accurate picture. And, while pregnancy detection is important, in keeping with our research group's concern for the safety and humane treatment of horses, **remote** pregnancy testing is an integral part of a complete hands-off approach to fertility control.

Experimental controls: Previous work with feral horses on Assateague Island national Seashore has documented the lack of contraceptive effects of placebo vaccination upon control animals. However, the validity of the proposed field test will be insured by including placebo controls for each type of treatment. The control preps will consist of an emulsion of phosphate buffer solution and Freund's adjuvant.

Treatment Evaluation: Field studies of contraception can be evaluated and measured for success or failure in different ways.

Our approach is to document the pharmacological success of contraception. This will be accomplished by comparing pregnancy and foaling rates among treated and untreated mares. This is a major focus of the present proposal and will be carried out by our research group. While it will ultimately be necessary to understand what the effects of contraception may be upon the population dynamics, this is beyond the scope of our proposed studies. Nonetheless, the proposed field trial can provide the beginning of a data base for population models to determine to what degree immunocontraception may alter the demographic dynamics and size of a feral horse herd.

Animal care: All research conducted in the course of this project will be subject to review by the appropriate animal research committees of the three institutions involved (Medical College of Ohio, Deaconess Research Institute, and the University of California at Davis), and will be conducted only after approval by these committees. The regulations surrounding animal care standards for wild or free-roaming species are not clear. However, our group will apply the standards for domestic animals to the treatment of all horses in this study, whether domestic or free-roaming.

Education and public relations: Our research group's experience with the highly visible and successful Assateague Island feral horse contraception study has made it extremely clear that a serious attempt must be made to keep the public informed and to provide open and honest dialogue with the media. The Assateague horses are the most visible - and perhaps most adored - feral horses in North America, and embarking upon the immunocontraceptive research project carried with it a certain amount of risk. In order to keep the public informed at each step of the project, the National Park Service conducted an extensive educational program. This involved the print media, local and national network TV, and on-site programs. After six years of research with this highly visible herd, which has some 700,000 visitors come to view it each year, there has been absolutely no public resistance and overwhelming public support, including animal protection groups. The key elements of this successful relationship with the public were careful documentation of each step of the research and willingness and efforts to share this information with the public. It is our intention to do the same thing with this proposed research. An experienced public relations expert will be retained by the research team on a consulting basis, to design an appropriate public relations program and to develop the necessary materials for disseminating information. Our research group has never killed or even seriously injured a horse in the course of 18 years of research; we are as proud of that as we are of our contraceptive success. We feel that the public must be able to view our work and the care we take if this approach to the control of feral animal populations is to become accepted. No information will be released without going through the consultant resource, who must have approval of the research team scientists for any information release.

INVESTIGATOR EXPERIENCE

The three investigators are Dr. John W. Turner, Jr., Department of Physiology and Biophysics, Medical College of Ohio, Toledo, Dr. Jay F. Kirkpatrick, Deaconess Research Institute, Billings, MT, and Dr. Irwin K. M. Liu, University of California, Davis, and the collaborating agency is the Humane Society of the U.S. Drs. Turner and Kirkpatrick have been involved in studies of the biology of feral horses for 18 years. These studies have focused on hormonal contraception and immunocontraception of both stallions and mares and culminated in the successful immunocontraception of the Assateague horses. Funding for these projects have come from a variety of source but primarily from the Department of the Interior, through the Bureau of Land Management (Contract YA-512-CT) and the National Park Service (Contract CA-1600-30005). In addition to contraceptive studies these two investigators have also pioneered non-capture methodologies for detecting pregnancy and monitoring ovarian function among free-roaming feral horses in order to develop a complete "hands-off" technology for the control of feral horse reproduction. Both investigators will personally devote a significant portion of their time to this project. Specifically, Dr. Turner will oversee the chemical engineering of the one-inoculation vaccine and play a significant role in designing and conducting the field testing of the vaccine. Dr. Kirkpatrick will be in charge of remote pregnancy detection, evaluation of vaccine delivery equipment, development of the public relations program and will participate in field tests. Together these investigators are responsible for 28 published scientific articles relating to feral horse biology and contraception, as well as numerous articles in the popular press. Dr. I.M.K. Liu is an equine immunologist in the School of Veterinary Medicine at U.C.-Davis. Dr. Liu was responsible for originally determining that the PZP vaccine is effective in horses and he has extensive experience testing this vaccine with feral horses living on sanctuaries. He will be in charge of vaccine production and antibody testing. All investigators will be present for the gathering and treatment of horses. Academic credentials and qualifications for the three co-investigators are provided in the appendix.

PROJECT EVALUATION

The project will be evaluated periodically at several check points, as well as at the conclusion. The check points, derived from the stated goals include (1) <u>in vivo</u> testing of the microsphere PZP vaccine (evaluation criteria = antibody concentrations and pregnancy rates), (2) <u>in vivo</u> testing of the microcapsule PZP vaccine (evaluation criteria = antibody concentrations and pregnancy rates), (3) effectiveness in the field of the vaccine delivered to feral horses percent of treated vs. control mares which produce foals. All endpoint evaluations are measurable and will result in data which can be tested for significance.

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Wang, H.T., H. Palmer, R.J. Linhardt, D.R. Glanagan, & E. Schmidt 1990. Degradation of poly(ester) microspheres. Biomaterial 11:679-685.

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PROPOSED PROJECT BUDGET

SECTION I. UNIVERSITY OF NEVADA, RENO (UNR) BUDGET

A. Personnel

Principal Investigator (K. Hunter) $5,831
(P.I. commitment to project is 5% of total time, plus 19% fringe benefits)

B. Travel

Travel from university to study site via university vehicle for P.I. and members of oversight committee $500

DIRECT COST TOTAL	$6,331
INDIRECT COST TOTAL*	$11,075
TOTAL UNR COSTS	$17,406

* includes indirect costs on first $25,000 of subcontract to Medical College of Ohio

SECTION II. PROPOSED SUBCONTRACT BUDGET (MEDICAL COLLEGE OF OHIO)

PART I. Chemical Engineering (Microsphere/Microencapsulation) Study

A. Personnel

Principal Investigator (J. Turner) $14,523.00
(P.I. commitment to this project is 20% of full-time effort. Plus 34% fringe benefits)

Co-Principal Investigator (J. Kirkpatrick) $ 6,100.00
(Co-P.I. commitment to this project is 10% of full-time effort. Plus 22% fringe benefits)

Research Associate $18,000.00
(Salary for preparation of PZP. 30% of full-time effort. Plus 34% fringe benefits)

Laboratory/Secretarial Assistance $14,472.00
(Part-time, $9/hr. X 24 hrs/wk (Medical College of Ohio) x 40 wks, plus 34% fringe benefits)

Laboratory Technician $16,080.00
(Part-time, $10/hr. X 30 hrs/wk X 40 wks, plus 34% fringe benefits)

SUBTOTAL $69,175.00

B. **Microsphere and Microcapsule Formulation and Testing**

Viability testing of vaccine for the formulations	$ 5,000.00
Timed-release vaccine preparation	$16,000.00
Vaccine release characteristics testing	$14,000.00
In vivo testing of the timed-release vaccine	$15,000.00
SUBTOTAL	$50,000.00

C. **Equipment**

Dionex Pulsed Electrochemical Detector and electrode for HPLC analysis of urine/feces	$ 7,900.00
Reciprocal shaker for urine/fecal extractions	$ 2,000.00
SUBTOTAL	$ 9,900.00

D. **Supplies**

Supplies for PZP preparation, antibody monitoring, blood collection, horse maintenance	$ 5,800.00

E. **Communications**

Phone, fax, mailing, copying	$ 1,600.00

F. **Consultants**

Public Relations Costs	$ 6,000.00

G. **Travel**

Principal Investigator (J. Turner):	
Toledo to site for microsphere preparation	$ 900.00
Toledo to site for microencapsulation preparation (2 trips)	$ 1,800.00
Toledo to site for timed-release vaccine testing in vivo	$ 900.00
SUBTOTAL	$ 17,000.00

DIRECT SUBCONTRACT COST SUM (PART I)	$146,075.00
MCO Indirect Costs (20% of above)	$ 29,215.00
TOTAL SUBCONTRACT COSTS (PART I)	**$175,290.00**

Part II. Field Trials Study

The costs of field trials will depend on the range site selected. Since conditions and tactical support elements vary

considerable from range to range, it is not possible to make a reliable cost projection. However, there are some aspects of the field trial costs which are fixed and an overall cost estimate can be made, assuming up to 140 mares will be treated.

The following budget is divided into 2 parts. Section A shows costs which will be provided to the Medical College of Ohio, and Section B shows costs which will be covered within the operating budget of the BLM.

Section A. (Costs Provided to MCO)

1. Personnel costs for 2 field technicians (students) to carry out the field monitoring of the PZP-treated and placebo horses, including urine/fecal sample collections for pregnancy testing and behavioral monitoring. Vehicles, fuel, and housing to be provided by BLM.

   $8.00/hr X 8 hr/day X 100 days X 2 persons (plus 34% fringe benefits) $ 17,152.00

   $15.00/person per diem X 100 days $ 3,000.00

   SUBTOTAL $ 20,152.00

2. Equipment

   a. Horse identification by videotape has proven superior in our studies, and we recommend that each monitoring person have such capability. Cost for freeze-frame videocamera is about $1,500.
   Sony TR-101 handycam $1,500 X 2 $ 3,000.00

   b. Binoculars $200 X 2 $ 400.00

   c. Spotting scope $300 X 2 $ 600.00

   SUBTOTAL $ 4,000.00

3. Supplies and communications, i.e., for sample collection and storage, horse monitoring, phone and mailing $ 2,500.00

4. The cost of vaccine will depend on the results of the Microsphere-Microencapsulation study and on the number of horses to be treated. A conservative estimate is $35/horse. If the experimental phase is successful a larger scale PZP preparation system will greatly reduce the cost per horse.
   Based on 110 mares treated plus 35 reserve doses.

Estimated Subtotal	$ 5,075.00

5. Cost of pregnancy testing will be approximately $15.00 per sample including shipping and assay and will be based on 140 mares (30 control and 110 experimental)
Estimated Subtotal $ 2,100.00

SUBTOTAL $ 9,675.00

6. Travel

Travel by Turner, Kirkpatrick, Liu and assistant to range site to perform vaccinations. $ 3,000.00

Travel by Dr. Turner or Kirkpatrick to verify foal counts and evaluate horse population in study range. $ 2,000.00

Travel by 2 field technicians to range site. $ 2,000.00

SUBTOTAL $ 7,000.00

Total Direct Costs for Section A.	$40,827.00
MCO Indirect Costs (20%)	$ 8,165.00
Total Costs for Section A.	**$48,992.00**

Section B. (Costs Covered Directly by BLM)

1. All helicopter costs: for initial observations of range, gathers of horses for PZP treatment and post-treatment monitoring (including flyovers for horses identifications and foal counts).

2. All equipment, supplies and personnel costs for gathering of horses and maintenance of captive horses, including corrals, freeze-branding, disease testing, veterinary care, feed, water/feed transport.

3. Provision of 4 X 4 vehicles and fuel for all research activities during the field trial.

PROJECT BUDGET SUMMARY

University of Nevada, Reno Costs	**$17,406**
Subcontract Costs (Medical College of Ohio)	**$224,282**
Total Project Costs	**$241,688**

CURRICULUM VITAE

Kenneth W. Hunter, Jr.

3460 Southampton Drive
Reno, Nevada 89509
(702) 324-1815

Present Positions:

Associate Vice President for Research
and Dean of the Graduate School
University of Nevada, Reno
239 Getchell Library
Reno, Nevada 89557-0035
Tel: (702) 784-6869
FAX: (702) 784-6064
e-mail: *khunter@unssun.nevada.edu*

Professor of Microbiology
University of Nevada School of Medicine
Department of Microbiology

Professor of Biology
University of Nevada, Reno
College of Arts & Sciences
Department of Biology

Recent Past Positions:

1986-1989	President and Chief Executive Officer Biotronic Systems Corporation Rockville, Maryland 20850 (Presently, Chairman of the Board)
1985-1989	Chief Scientist Westinghouse Bio-Analytic Systems Co. Madison, Pennsylvania
1982-1989	Founder and Executive Vice President ANTECH Consultants, Inc. Rockville, Maryland

Education:

1977	The Johns Hopkins University School of Hygiene and Public Health Department of Pathobiology Sc. D., Immunology-Parasitology
1972/73	Arizona State University Department of Zoology B.A., Biology M.S., Zoology

Academic Experience:

1986-89	Adjunct Associate Professor Uniformed Services University of the Health Sciences F. Edward Hebert School of Medicine Departments of Pediatrics and Preventive Medicine/Biometrics Bethesda, Maryland
1982-86	Associate Professor of Pediatrics and Preventive Medicine/Biometrics Director of Pediatric Research Uniformed Services University of the Health Sciences F. Edward Hebert School of Medicine Department of Pediatrics
1979-82	Research Assistant Professor (Primary Appointment) Uniformed Services University of the Health Sciences F. Edward Hebert School of Medicine Department of Pediatrics (Infectious Disease Section)
1978-79	Post-Doctoral Fellowship Uniformed Services University of the Health Sciences F. Edward Hebert School of Medicine

Graduate Grants and Fellowships::

1976	Visiting Research Associate Agency for International Development Malaria Research Project University of New Mexico Albuquerque, New Mexico
1975	Immunology Research Grant The John W. Graham Fund The Johns Hopkins University
1974-1977	Pre-Doctoral Fellowship Parasitology-Medical Entomology National Institutes of Health National Institute of Allergy and Infectious Diseases
1973	Research Collaboratorship United States Department of Agriculture Agriculture Research Service Western Cotton Research Laboratory Division of Entomology Phoenix, Arizona

Professional Societies::

American Association for the Advancement of Science
American Association of Immunologists
Helminthological Society of Washington
Tropical Medicine Association of Washington
American Society of Tropical Medicine and Hygiene
American Society of Clinical Pathology
Association of Official Analytical Chemists

University Committees:

Uniformed Services University of the Health Sciences (USUHS) Biohazard Suite Coordinating Committee, Chairman
Armed Forces Radiobiology Research Institute (AFRRI)/USUHS Radionuclide and X-Ray Safety Committee
USUHS Research Proposal Merit Review Committee
USUHS Faculty Senate Research Policy Committee
University of Nevada, Reno (UNR) Biomedical Human Subjects Committee
UNR Social/Behavioral Human Subjects Committee
UNR Biohazards, Controlled Substances, and Dangerous Materials Committee, (Recombinant DNA Subcommittee)
UNR Intellectual Property Committee
UNR Institutional Animal Care and Use Committee
UNR Radiation Safety Advisory Board
UNR Graduate Council
University and Community College System of Nevada Research Affairs Committee

UNR Representative To:

Western Association of Graduate Schools
National Association of State Universities and Land Grant Colleges, Council on Research Policy and Graduate Education
Intermountain University Research Administrators
Council of Graduate Schools
Council on Government Relations
Society of Research Administrators

Advisory Groups:

Technical Consultant, NSTA/NASA
Space Shuttle Student Involvement Project
Goddard Space Flight Center
Greenbelt, MD, 1984

Scientific Advisory Panel
European Journal of Epidemiology, 1984-

Advisory Panel
U.S. Environmental Protection Agency
Biomarkers Peer Review and Panel, 1986

Governor's Task Force on
Regional Economic Development
Greater Washington Region, 1988

The Johns Hopkins University Society of Alumni
Career Opportunities Committee, 1988

Advisory Panel
Congress of the United States
Office of Technology Assessment
"Technologies to Detect Pesticide
Residues in Food", 1988

Member, Board of Directors
Montgomery County High Technology
Council, Inc. 1988-1991

College of American Pathologists
Future Technology Committee, 1988-1989

American Society of Clinical Pathologists
New Technology Committee, 1988-1989

Elected Advocate
Maryland Conference on Small Business
1988-1990

State of Maryland, Governor's Office
Partnership for Workforce Quality
Advisory Board, 1989

The Johns Hopkins University
Montgomery County Center
Advisory Board, 1989

Nevada Innovation, Technology,
and Entreprenurial Council
Member, Board of Trustees, 1989-
Vice President, 1990-

Western Industrial Nevada
Member, 1990-

Nevada Industry, Science, Engineering
and Technology Organization, Inc.
Member, Board of Trustees, 1990-

Nevada State Development Corporation
Board of Trustees, 1991-

Nevada Space Grant
Associate Director, 1991-

Nevada State EPSCoR Committee
Member, 1990-

Research Interests:

Gene Regulation in Leishmania
Immunoregulatory Functions in Malaria
Medical and Agricultural Entomology
Monoclonal Antibodies for Chemical Haptens
Somatic Cell Genetics and Human Hybridomas
Biosensors and Molecular Electronics

Past Research Support:

K.W. Hunter, Principal Investigator
Temperature-Induced Transformation in Leishmania
USUHS RDT&E Grant
February 1980 - February 1982
$102,732

K.W. Hunter, Principal Investigator
Detection of Organophosphates Using Immunologic Methods
U.S. Army (AARADCOM) Chemical Systems Laboratory
May 1981 - September 1985
$599,000

K.W. Hunter, Principal Investigator
Human Monoclonal Antibodies to Malarial Antigens
U.S. Agency for International Development (USAID)
July 1981 - January 1983
$35,000

K.W. Hunter, Principal Investigator
Somatic Cell Cloning of Mouse and Human
Somanase and Butyrylcholinesterase

U.S. Army Chemical Research and Development Center
March 1983 - March 1985
$200,000

K.W. Hunter, Principal Investigator
Evaluation of the Prophylactic Potential of Monoclonal Anti-Soman Antibodies
U.S. Army Medical Research & Development Command
May 1983 - September 1986
$526,000

K.W. Hunter, Principal Investigator
Preparation and Characterization of Mouse and Human Monoclonal Antibodies to Botulinum Toxins
U.S. Army Medical Research & Development Command
April 1982 - September 1984
$136,000

K.W. Hunter, Principal Investigator
Rapid Biosensor Assay for AIDS Virus Antibodies
National Institute of Allergy and Infectious Disease
Small Business Innovation Research Program
February 1989-June 1990 : Phase I $50,000
June 1990- : Phase II $500,000

Awards:

USUHS Outstanding Performance Awards, 1979-1985
Department of the Army, Special Commendation, 1984
USUHS Distinguished Service Medal, 1987
Who's Who in Science and Technology, 1991

Courses Taught:

Diagnostic Parasitology and Medical Zoology
Medical Microbiology (Immunology and Parasitology)
Medical Entomology
Epidemiology
Preventive Medicine and Public Health

PUBLICATIONS

1. **Hunter, K.W.** 1973. Effect of the parasite *Copidosoma truncatellum* (Dalman) on food consumption of *Trichoplusia ni* (Hubner) larvae. Masters Thesis, Arizona State University, Tempe, Arizona.

2. **Hunter, K.W.** and A. Stoner. 1975. *Copdiosoma truncatellum*: Effect of parasitization on food consumption of *Trichoplusia ni*. Environ. Entomol. 4:381-382.

3. **Hunter, K.W.** and A.C. Bartlett. 1975. Chromosome number of the parasitic encyrtid *Copidosoma truncatellum* (Dalman). Ann. Entomol. Soc. Amer. 68:61-61.

4. **Hunter, K.W.** 1977. Serum opsonic activity in rodent malaria. Doctoral Thesis, The Johns Hopkins University, Baltimore, MD.

5. **Hunter, K.W.** 1979. Searching behavior of *Hippodamia convergens* larvae (Coccinellidae: Coleoptera). Psyche 85:249-254.

6. **Hunter, K.W.**, F.D. Finkelman, G.T. Strickland, P.C. Sayles,and I. Scher. 1979. Defective resistance to *Plasmodium yoelii* in CBA/N mice. J. Immunol. 123:133-137.

7. **Hunter, K.W.**, A.R. Campbell, and P.C. Sayles. 1979. Human infestation by cat fleas, *Ctenocephalides felis* (Siphonaptera: Pulicidae), from suburban raccoons. J. Med. Entomol. 16:547.

8. **Hunter, K.W.**, J.A. Winkelstein, and T.W. Simpson. 1979. Serum opsonic activity in rodent malaria: functional and immunochemical characteristics in vitro. J. Immunol. 123:2582-2587.

9. **Hunter, K.W.**, G.W. Fischer, P.C. Sayles, and G.T. Strickland. 1979. Increased resistance to malarial infection following treatment with the immunostimulator levamisole. Curr. Chemother. Infect. Dis. 2:1099-1101.

10. Mease, A.D., G.W. Fischer, **K.W. Hunter**, and F.B. Ruymann. 1980. Decreased phytohemagglutinin-induced aggregation and C5a-induced chemotaxis of newborn neutrophils. Pediat. Res. 14:142-146.

11. **Hunter, K.W.**, F.D. Finkelman, G.T. Strickland, P.C. Sayles, and I. Scher. 1980. Murine malaria: Analysis of erythrocyte surface-bound immunoglobulin by flow microfluorimetry. J. Immunol. 125:169-174.

12. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and A.D. Mease. 1980. Diminished bacterial defenses with intralipid. Lancet 2:8I9-820.

13. Strickland, G.T., and **K.W. Hunter**. 1980. The use of immunopotentiators in malaria. Int. J. Nuc. Med. Biol. 7:133-140.

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WILD HORSE AND BURRO FERTILITY MANAGEMENT

POLICY AND PROCEDURES TASK GROUP

FINAL REPORT

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Prepared by

DEPARTMENT OF INTERIOR
BUREAU OF LAND MANAGEMENT
NEVADA STATE OFFICE

Kenneth G. Walker, Chairman — Date: 6-30-92

Billy R. Templeton, State Director, Nevada — Date: 7/29/92

This Wild Horse and Burro Fertility Management Pilot Project Policy and Procedures Task Group Final Report recommends actions to be implemented in a pilot project evaluating contraceptive management in the Antelope and Antelope Valley herd management areas. It examines available options in contraceptive management and recommended monitoring procedures in evaluating the potential viability of that management in wild horse populations and in ensuring the humane treatment of animals.

For further information contact: Thomas Pogacnik, Wild Horse and Burro Specialist, Bureau of Land Management, Nevada State Office, 850 Harvard Way, P.O. Box 12000, Reno, Nevada 89520, or telephone (702) 785-6583.

14. Fischer, G.W., **K.W. Hunter**, and S.R. Wilson. 1980. Intralipid and reticuloendothelial clearance. Lancet 1:1300.

15. Strickland, G.T., and **K.W. Hunter**. Red cell antibodies in malaria: Immunity or Autoimmunity? *In* The Host-Invader Interplay, H. Van den Bossche ed., Elsevier/North-Holland Biomedical Press, Amsterdam, 1980.

16. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and S.A. Henson. The role of antibody in Group B streptococcal disease. *In* Immunoglobulins: Characteristics and Used of Intravenous Preparations. B.M. Alving, J.S. Finlayson, eds. Washington, D.C. U.S. Government Printing Office, 1980:DHEW publication No. (FDA)-80-9005.

17. **Hunter, K.W.**, T.M. Folks, P.C. Sayles, and G.T. Strickland. 1981. Early enhancement followed by suppression of natural killer cell activity during murine malarial infections. Immunol. Lett., 2:209-212.

18. Fischer, G.W., **K.W. Hunter**, S.R. Wilson, and V.G. Hemming. 1981. Modified immune serum globulin. Lancet 1:271.

19. **Hunter, K.W.**, G.W. Fischer, P.C. Sayles, and G.T. Strickland.1981. Levamisole: Potentiation of primary immunoglobulin M antibody responses in suckling rats. Immunopharmacology 3:117-127.

20. Sayles, P.C., **K.W. Hunter**, E.E. Stafford, and L.D. Hendricks. 1981. Antibody response to *Leishmania mexicana* in the African white-tailed rat (*Mystromys albicaudatus*). J. Parasitol. 67:585-586.

21. **Hunter, K.W.**, L.P. Smith, G.T. Strickland, and W.C. Blackburn. 1981. Hypergammaglobulinemia and erythrocyte autoantibody complicate enzyme immunoassay of antimalarial antibody. J. Immunoassay 2:99-108.

22. Fischer, G.W., **K.W. Hunter**, and S.R. Wilson. 1981. Type III Group B streptococcal strain differences in susceptibility to opsonization with human serum. Pediat. Res. 15:1525-1529.

23. **Hunter, K.W.**, and D.E. Lenz. 1982. Detection and quantification of the organophosphate insecticide paraoxon by competitive inhibition enzyme immunoassay. Life Sci. 30:355-361.

24. Fischer, G.W., S.R. Wilson, and **K.W. Hunter**. 1982. Functional characteristics of a modified immunoglobulin preparation for intravenous use: Summary of studies of opsonic and protective activity against Group B streptococci. J. Clin. Immunol., 8:325-365.

25. **Hunter, K.W.**, C.L. Cook, and S.A. Hensen. 1982. Temperature-induced in vitro transformation of *Leishmania mexicana*. I. Ultrastructural comparison of culture-transformed and intracellular amastigotes. Acta Trop. 39:143-150.

26. Fischer, G.W., **K. W. Hunter**, and S.R. Wilson. 1982. Modified human immune serum globulin for intravenous administration: Opsonic and protective effect against group B streptococci. Acta Pediat. Scand. 71:639-644.

27. **Hunter, K.W.** Separation and purification of blood stage malaria parasites. *In* Immunoparasitology: Principles and Methods in Schistosomiasis and Malaria Research, G.T. Strickland and K.W. Hunter, eds., Praeger Publishers, New York, 1982, pp. 208-211.

28. **Hunter, K.W.** Cell-mediated cytotoxicity: Measurement of murine natural killer cell activity during malarial infection. *In* Immunoparasitology: Principles and Methods in Schistosomiasis and Malaria Research, , G.T. Strickland and K.W. Hunter, eds., Praeger Publishers, New York, 1982, pp. 237-240.

29. Smith, L.P., and **K.W. Hunter**. Clearance and sequestration studies using radiolabelled *Plasmodium yoelii*-infected erythrocytes. *In* Immunoparasitology: Principles and Methods in Schistosomiasis and Malaria Research, G.T. Strickland and K.W. Hunter, eds., Praeger Publishers, New York, 1982, pp. 231-236.

30. **Hunter, K.W.** Application of mitogen-induced lymphocyte proliferation to the study of malarial immunosuppression. *In* Immunoparasitology: Principles and Methods in Schistosomiasis and Malaria Research, G.T. Strickland and K.W. Hunter, eds., Praeger Publishers, New York, 1982, pp. 241-245.

31. **Hunter, K.W.** Radioimmunoassay: Quantification of the antibody response to *Plasmodium yoelii* in mice. *In* Immunoparasitology: Principles and Methods in Schistosomiasis and Malaria Research, G.T. Strickland and K.W. Hunter, eds., Praeger Publishers, New York, 1982, pp. 246-250.

32. Smith, L.P., **K.W. Hunter**, E.C. Oldfield, and G.T. Strickland. 1982. Murine malaria: Blood clearance and organ sequestration of *Plasmodium yoelii*-infected erythrocytes. Infect. Immun., 39;162-167.

33. **Hunter, K.W.**, G.W. Fischer, V.G. Hemming, S.W. Wilson, R.J. Hartzman, and J.N. Woody. 1982. Antibacterial activity of a human monoclonal antibody to *Haemophilus influenzae* type b capsular polysaccharide. Lancet, 2:798-799.

34. **Hunter, K.W.**, D.E. Lenz, A.A. Brimfield, and J.A. Naylor. 1982. Quantification of the organophosphorus nerve agent soman by competitive inhibition enzyme immunoassay using monoclonal antibody. FEBS Lett.. 149:147-151.

35. Fischer, G.W., **K.W. Hunter**, and V.G. Hemming. 1983. Human monoclonal antibodies for prophylaxis and therapy of infectious diseases. Infect. Dis. 13:4-25.

36. **Hunter, K.W.**, C.L. Cook, and E.E. Stafford. 1983. *Leishmania mexicana*: Analysis of amastigote and promastigote antigens by crossed immunoelectrophoresis. Trans. Roy. Soc. Trop. Med. Hyg. 77:177-180.

37. Fischer, G.W., **K.W. Hunter**, V.G. Hemming, and S.R. Wilson. 1983. Functional antibacterial activity of a human intravenous immunoglobulin preparation. In vitro and in vivo studies. Vox Sang. 44:296-299.

38. Bosworth, Jr., J.M., A.A. Brimfield, J.A. Naylor and **K.W. Hunter**. 1983. Measurement of monoclonal antibody concentration in hybridoma cultures. Comparison of competitive inhibition and antigen capture enzyme immunoassays. J. Immunol. Meth. 62:331-336.

39. Strickland, G.T., A. Ahmed, P.C. Sayles, and **K.W. Hunter**. 1983. Murine malaria: Cellular interactions in the immune response. Amer. J. Trop. Med. Hyg. 23:1229-1235.

40. Pollack, M., A.I. Huang, R.K. Prescott, L.S. Young, **K.W. Hunter**, D.F. Cruess, and C.M. Tsai. 1983. Enhanced survival in *Pseudomonas aeruginosa* septicemia associated with high levels of circulating antibody to *Escherichia coli* endotoxin core. J. Clin. Invest. 72:1874-81.

41. Smith, L.P., **K.W. Hunter**, V.G. Hemming, and G.W. Fischer. 1983. Improved detection of bacterial antigens by latex agglutination after rapid extraction from body fluids. J. Clin. Microbiol. 20:981-984.

42. Lenz, D.E., A.A. Brimfield, **K.W. Hunter**, H.P. Benschop, L.P.A. de Jong and T.R. Clow. 1984. Studies with monoclonal antibodies to soman. Fund. Appl. Toxicol. 4:S156-S164.

43. Fischer, G.W., L.B. Weisman, V.G. Hemming, W.T. London, **K.W. Hunter**, J.M. Bosworth, J.L. Sever, S.R. Wilson, and B.L. Curfman. 1984. Intravenous immunoglobulin in neonatal group B streptococcal disease: Pharmacokinetic and safety studies in monkeys and humans. Amer. J. Med. 76:117-123.

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SPECIAL INVITED PRESENTATIONS

United States Army Biomedical Laboratory Symposium, The Immunology of Organophosphorus Compounds, Aberdeen Proving Ground, MD, May 20, 1981. Presentation entitled, *"The Use of Monoclonal Antibodies and Enzyme Immunoassay for Detection of Organophosphorus Compounds".*

First Annual U.S. Army Armament Research and Development Command Technical Conference, Picatinny Arsenal, Dover, NJ, June 16-18, 1981. Presentation entitled, *"Detection of Paraoxon".*

1981 Chemical Systems Laboratory Scientific Conference on Chemical Defense Research. Aberdeen Proving Ground, MD, 16-20 November 1981. Presentation entitled, *"Immunologic Method for the Rapid Detection of Soman (GD)".*

Research Colloquium, Chemical Systems Laboratory, Aberdeen Proving Ground, MD., July 9, 1982. Presentation entitled, *"Immunodetection of Soman".*

1982 Chemical Systems Laboratory Scientific Conference on Chemical Defense Research, Aberdeen Proving Ground, MD, 15-18 November 1982. Presentation entitled, *"Inhibition of the Anticholinesterase Activity of Soman by Monoclonal Antibody".*

1983 International Symposium on Protection Against Chemical Warfare Agents, Stockholm, Sweden, June 6-9, 1983. Presentation (A.A. Brimfield) entitled, *"Fine Specificity of Anti-Soman Monoclonal Antibodies".*

Research Colloquim. Chemical Research and Development Center, Aberdeen Proving Ground, MD, October 7, 1983. Presentation entitled, *"Immunodetection of the Trichothecene Mycotoxin T-2".*

European Symposium on "New Horizons in Microbiology", April 26-29, 1984. Presentation entitled,*"Protection Against Haemophilus influenzae type B by Antibodies to the Capsular Polysaccharide".*

1984 FASEB Summer Research Conference on Diagnosis, Toxicity, and Therapy of Trichothecene Mycotoxicosis, June 25-29, Vermont Academy, Saxtons River, VT. Presentation entitled, *"Immunodetection of T-2 Toxin Using Monoclonal Antibody".*

1984 Chemical Research and Development Center Scientific Conference on Chemical Defense Research. Aberdeen Proving Ground, MD, 12-16 November 1984. Presentation (R.F. Schuman) entitled, *"Secretion of Acetylcholinesterase by a Hybrid Cell Line".*

USUHS Continuing Medical Education, Japan and Okinawa Tour, May 1984. *"Presentations on Advances in Medical Biotechnology and Tropical Medicine".*

U.S. Environmental Protection Agency, Peer Review and Biomarkers Panel Meeting, San Jose, CA. June 24-26, 1986. Presentation entitled, *"Detection of Chemical Warfare Agents Using Monoclonal Antibody-Based Immunoassays".*

Research Colloquium: New Methods in the Detection of Environmentally Important Compounds. U.S. Environmental Protection Agency, Research Triangle Park, NC. November 20-21, 1986. Presentation entitled, *"Monoclonal Antibody-Based Immunoassay for Toxic Chemicals".*

IEEE Conference on Synthetic Microstructures in Biological Research, Airlie, VA. March 23-26, 1986. Presentation (A.L. Newman) entitled, *"Development of an Antibody-Modulated Planar Capacitive Sensor".*

College of American Pathologists, 1987. Annual Meeting, Molokai, Hawaii. January 15-18, 1987. Presentation entitled, *"Technological Advances in Bedside Monitoring: Biosensors".*

National Food Processors Association, Atlantic City, NJ, March 12, 1987. Presentation entitled, *"Monoclonal Antibody-Based Immunoassays for Toxic Organic Compounds".*

American Chemical Society, Division of Agrochemicals Special Conference III, Biotechnology in Crop Protection, June 28-July 3, 1987, Snowbird, Utah, Presentation (W.L. Stanbro) entitled, *"Interfacing of Hybridoma and Microelectronics for Use in Environmental Analysis".*

American Pathology Foundation, 10th Annual Meeting, July 11-14, 1987, San Diego, CA. Presentation entitled, *"Biosensors and Telemetry for Near Instantaneous Viral Diagnosis: Hepatitis and Acquired Immune Deficiency Syndrome".*

Society for Industrial Microbiology, Annual Meeting (August 9-15, 1987, Baltimore, MD. Presentation entitled, *"Capacitive Affinity Sensor: A New Multi-Purpose Biosensor".*

EPRI PCB Seminar, October 6-9, 1987, Kansas City, MO. Presentation (R.F. Schuman) entitled, *"Ultrasensitive Bioassay for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)".*

American Pathology Foundation Seminar "Managing for Profit: Taking Advantage of New Techniques and Emerging Opportunities in Pathology", February 26-27, 1988, Charleston, SC. Presentation entitled, *"Biosensors and Telemetry in Bedside Testing".*

American Society of Clinical Pathology Symposium: Present and Future Applications of New Technology in Anatomical and Clinical Pathology, April 18, 1988, Kansas City, MO. Presentation entitled, *"Advances in Bedside Monitoring: Biosensors".*

Association of Official Analytical Chemists Biotechnology Symposium, August 29 September 1, 1988. Palm Beach, FL. Presentation entitled, *"Biosensors: A New Dimension for Immunoassays".*

Symposium: Developments in Biosensor Technology, July 25, 1988, New Orleans, LA. Presentation entitled, *"Capacitive Affinity Sensors: Multi-Purpose Biosensors"*.

International Biosensors '88 Symposium, August 22-23, 1988, Washington, D.C. Presentation entitled, *"Commercializing Biosensors: The Transition from the Research Laboratory to the Marketplace"*.

American Society of Clinical Pathologists/College of American Pathologists Spring Meeting, March 11-16, 1989, Chicago, IL. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classification and Evolution"*.

International Biosensors '89 Symposium, October 16-17, 1989, New Orleans, LA., Presentation entitled, *"Identifying Market Niches for Biosensors"*.

American Society of Clinical Pathologists/College of American Pathologists Fall Meeting, October 28-November 3, 1989, Washington, D.C. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classification and Evolution"*.

American Society of Clinical Pathologists/College of American Pathologists Fall Meeting, October 20-24, 1990, Dallas, TX. Symposium on Biosensors in the Practice of Medicine, presentation entitled, *"Classification and Evolution"*.

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